UTILISATION OF *Moringa oleifera* LAM. SEED AS PROTEIN SOURCE IN THE DIETS OF BROILER CHICKENS

BY

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ABSTRACT

Conventional vegetable protein ingredients in poultry feed are costly and relatively scarce. *Moringa oleifera* Seed Meal (MSM) is an oil seed rich in protein, which can be exploited as an alternative vegetable protein source. However, the potential of MSM as a protein source for broiler production has not been adequately documented. Therefore, this study was conducted to investigate the utilisation of MSM as a protein source in the diets of broiler chickens.

Moringa seeds were harvested and apportioned into four parts representing T1, T2, T3 and T4. The T1 was raw, while T2, T3 and T4 were soaked in water (200 g/L) for 1, 2, and 3 hours, respectively. The seeds were then sun-dried, milled and analysed for Crude Protein (CP, %), alkaloid (%), saponin (%) and tannin (%), using standard procedures. The protein quality of MSM was assessed using forty weanling albino rats (42.35±0.83 g) randomly allotted to four diets containing 10.0% casein (D1), 10.0% raw MSM (D2), 10.0% 3-hour Water-soaked MSM (WMSM, D3) and a protein free diet (D4) fed for 21 days. Protein quality: Biological Value (BV, g), Protein Efficiency Ratio (PER) and Net Protein Utilisation (NPU, g) were determined using standard procedures. Two hundred and fifty 1-day old broiler chicks were randomly allotted to five diets wherein Full-fat Soyabean (FS) was replaced with 3-hour WMSM at 0.0, 25.0, 50.0, 75.0 and 100.0%. Daily Weight Gain (DWG, g/bird), Feed Intake (FI, g/bird), and Feed Conversion Ratio (FCR) were measured. Blood (5mL) samples were collected for determination of Packed Cell Volume (PCV, %), Red Blood Cell (RBC $x10^6$/µL) and White Blood Cell (WBC$x10^3$/µL) counts, Total Cholesterol (TC, mg/dL), triglycerides (mg/dL) and immunoglobulins: IgG, IgA and IgM (IU/dL) using standard procedures. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

The CP of T1 (35.4±0.7), T2 (35.3±0.4), T3 (35.7±0.8) and T4 (36.1±0.7%) were similar, while alkaloid, saponin and tannin content ranged from 0.05±0.10 (T4) to 0.07±0.04 (T1), 0.42±0.50 (T4) to 1.17±0.28 (T1) and 0.05±0.02 (T4) to 0.06±0.02 (T1), respectively. The BV ranged from 54.87±5.54 (D4) to 65.69±5.70 (D1), PER from 0.63±0.08 (D4) to 1.10±0.33 (D1) and NPU from 15.86±1.22 (D4) to 44.37±4.31 (D1). The birds fed 100.0%WMSM had least DWG (0.63±0.11), while birds on 100.0%FS had the highest (1.30±0.15). The FI was least in 100.0%WMSM (2.19±0.86) and highest in 100.0%FS (30.00±7.86). The FCR ranged from 2.45±0.32 (100.0%FS) to 3.48±0.26 (100.0%WMSM). The PCV ranged from 29.7±0.54 (100.0%FS) to 34.5±0.86 (100.0%WMSM), RBC from 2.98±0.06 (75.0%WMSM) to 3.44±0.08 (25.0%WMSM) and WBC from 1.25±0.52 (100.0%FS) to 1.53±0.64 (100.0%WMSM). Birds fed 100.0%WMSM had least TC (157.67±10.72) compared with 100.0%FS (206.33±13.52) and triglycerides, which ranged from 56.00±3.26 (100.0%WMSM) to 85.36±5.35 (100.0%FS). The IgG, IgA and IgM recorded for birds on WMSM diets ranged from 0.62±0.36 (100.0%FS) to 0.73±0.40 (100.0%WMSM), 0.31±0.18 (100.0%FS) to 0.49±0.15 (100.0%WMSM) and 0.09±0.04 (100.0%FS) to 0.15±0.08 (100.0%WMSM), respectively.

Three hours water-soaked *Moringa oleifera* seed meal at 100.0% inclusion reduced growth, enhanced immunoglobulins profile and lowered total cholesterol of broiler chickens.

Keywords: *Moringa oleifera*, Full-fat soyabean, Protein quality, Immunoglobulins.

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CERTIFICATION

I certify that this work was carried out by Mrs Esther Eghosa AKANGBE in the Department of Animal Science, University of Ibadan

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DEDICATION

This work is dedicated to the glory of God Almighty who has given me the support, confidence, boldness, guidance, opportunity and glory in doing this research work and to my late parents, Mr. and Mrs. Emmanuel Clara Oviawe.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

CHAPTER ONE

INTRODUCTION

Animals and humans depend on the ingestion of minimum daily quantities of certain amino acid constituents of protein designated as the "essential or indispensable" amino acids (Ajenifuja, 1987). This term refers to a group of amino acids which cannot be synthesised in the organism at all or at a rate adequate to meet its metabolic requirements. Ingested protein is enzymically hydrolysed to the constituent amino acids as an initial metabolic step in animals and humans (Aletor *et al*., 2000).

Protein is one of the critical nutrients particularly for young growing animals and humans for high production and development. The products of digestion of dietary proteins are mainly carried by the blood as free amino acids. Their concentration in each tissue is the result of a balance between the input from the blood supply, catabolism of protein, the loss due to protein synthesis and some catabolic pathways such as the release of energy from free amino acids in the blood pool. The protoplasm is mainly protein and protoplasm is the control centre of a living cell which is the basic unit of life. Proteins are thus the most abundant macromolecules in the body. They are indispensable constituents of blood, muscles, organs, skins, tendons, bones, nails, and feathers and explain in part the versatility in the functions they perform (Durrani *et al*., 2006).

Protein is particularly important in growth, replacement and repair of worn-out tissues, because of the structural functions of the protein found in cyto-skeletal structures (Endoplasmic reticulum) and extracellular matrix of tissues and in muscles. Protein also furnish carbon skeletons for the production of energy. While there appears to be no evidence of direct conversion to fat, it has long been established that conversion of protein to carbohydrate is

substantial. Evidence of glucogenic nature of amino acids has been demonstrated which permits nearly all amino acids to be converted to intermediates such as pyruvate, oxaloacetate, propionyl CoA or α-ketoglutarate, all of which are known to be convertible to glucose (Durrani *et al.*, 2006). This tends to establish the idea that the usefulness of protein and amino acids in supplying energy to the body is not only limited to severe conditions of starvation but occurs in a day to day metabolic process. This is further enhanced if the mixture of protein in the feed is not supplying the level of each amino acid required for growth and maintenance purposes, in which case excess amino acids in the blood pool as a result of the imbalance are oxidized to release energy since amino acids are not stored in the body in appreciable amounts.

An important characteristic of protein is that most enzymes are protein, although many enzymes employ co-factors or prosthetic groups of a non-protein nature (Bunyan and Price, 1992). It is now agreed that should such a substance be found, it will not be called an enzyme. It has also been shown that the immune response of animals and man are higher if they are fed rations that are adequate in protein. However, the importance of other components of the feed should be stressed because the nutritive value of the protein content of the diet is affected by its energy value, digestibility, rancidity of associated fats and most especially the presence and availability of vitamins and minerals that accompany protein in a diet (Madubuike 1992).

Despite the importance of protein in the body, there is still a deficit of food protein intake in the great majority of livestock, (WHO 1981, Aletor 2000). The widespread nature of malnutrition and under-nutrition has been a long-term concern of developing countries, coupled with the problem of inadequate and inefficient distribution channels usually prevalent in these countries. The inadequate feeds problem has forced governments of developing countries and some private organisations to become involved in the development and commercialisation of low-cost, high-nutrition feed products for consumption by animals.

In West Africa, there is a general need for readily available, high quality, alternative vegetable protein and energy sources that are inexpensive and capable of reducing production costs of meat

and other animal products. This would reduce protein-calorie malnutrition and also help to alleviate poverty in the region (Tewe and Ologhobo, 2001).

Poultry and piggery have been long recognized as domestic livestock exhibiting rapid growth. Products derivable from poultry production ranges from major product like egg and meat to by-product such as; feathers, intestinal organs and bones.

An important factor in the continued growth of the poultry industry among others is the perceived health value of poultry meat in human diets which can only be achieved in disease-free birds (Kruchten, 2009). However, commercial poultry possess limited natural resistance and immunity against colonization or infection by pathogenic microorganisms. Thus, antimicrobial feed additives have made a tremendous contribution to profitability of intensive husbandry and provision of nutritious poultry products.

As a result of this, development of alternative antimicrobial agents that will improve the production of livestock and will not have residual effect on livestock is of great importance (Ogbe and Affiku, 2012). *Moringa oleifera* seed and full-fat soyabean are important in poultry nutrition, therefore this study was conducted to explore the utilisation of *Moringa oleifera* seeds as alternative to full-fat soyabean in the diets of broiler chickens.

Moringa seed kernels contain a significant amount of oil that commercially known as “Ben oil” or “Behen oil”. The Ben oil was erroneously reported to be resistant to rancidity and used extensively in the “enfluerage” process (Ndabigengeser and Narasiah, 1998). Moringa seed oil content and its properties show a wide variation depending mainly on the species and environmental conditions (Ibrahim *et al.*, 2004).

In Pakistan, only two species of *Moringa: M. concanensis* and *M. oleifera* are reported. The former species is rare and confined to only a remote locality of Therparkar, Sindh province of Pakistan The latter species is mostly cultivated in the Sindh province and irrigated plains of the country (Oaiser, 1973; Manzoor *et al*., 2007).

Wild *M. oleifera* grows naturally in forests of North West Frontier Province of Pakistan. It is recognised as one of the main non-wood forest products of Pakistan. The wild fruit is collected by graziers from forests and sold in the local market. There has been report on a small supply of about 10 tons, which is used domestically (Iqbal, 1991).

The increasing human population pressure together with the currently growing momentum of oleo-chemicals and oils/fats derived fuels (Biodiesel) has made it imperative to take advantages of more and more vegetable oils and protein source to meet the growing needs of the livestock industries. Although a number of under explored plants have been identified, lack of information on their chemical composition has limited their applications. The detailed scientific knowledge regarding the composition of *M. oleifera* (wild type) seed oil is of considerable importance for the development and commercialisation of this potential protein source crop.

GENERAL OBJECTIVE OF THE STUDY

This study was undertaken:

- To evaluate the potential of *Moringa oleifera* seed meal in broiler production

Specific objectives

- To determined the chemical characterisation of raw and water-soaked *Moringa oleifera* seeds
- To evaluate the dietary protein quality of raw and water-soaked *Moringa oleifera* seed meal in albino rats
- To evaluate the effect of substituting full-fat soyabean for *Moringa oleifera* seed meal in broiler chickens

JUSTIFICATION OF THE STUDY

- *Moringa oleifera* seed is high in protein, hence, could be utilised as an alternative protein source in feed ingredient in poultry diet
- Since *Moringa oleifera* seed possesses anti nutritional properties, there is the need to study the effect of processing on its nutritive value in broilers' diet
- The use of *Moringa oleifera* seed in poultry diet can improve their performance

CHAPTER TWO

LITERATURE REVIEW

2.1 Nutrient requirements of the domestic chickens

The establishment of a substance as an essential nutrient for any animal depends on the demonstration of adverse effects on the animal in the absence of that substance from the diet. The nutrients required for metabolic and productive activities differ between classes of poultry and vary with age, sex, environment for the same species of bird (Fetuga, 1984; NRC, 1994). Also genetic differences exist, even within species, in quantitative requirements for individual nutrients (Brown, 2009). The nutrient requirement of poultry, particularly broiler chicks and laying hens are defined more precisely than for other domestic animals (Jordan and Petterson 2008). This is so because of the nature of the birds and the rather specific conditions under which they are produced and the short period of time involved, particularly for broilers, allow the development of much more information for a given cost than with other species (Castaldo, 2002).

All chickens, qualitatively, have the same nutritional requirements. They need about 40 nutrients; these consists of 13 important amino acids, 13 vitamins, 13 essential inorganic element, linoleic acid and sufficient non-specific nitrogen to allow for production of the non-essential amino-acids, and enough metabolisable energy (ME) to meet the energy needs for maintenance and production (Gupta *et al*., 2008). It is this need for energy that varies from one environment to another; and the energy requirements set the base for the qualitative requirements for all the other nutrients.

The needs of broiler chicks are considered more critically and extensively in formulating rations than for layers and close scrutiny is therefore given to virtually all nutrients for which requirements data are available. With adult layers for either commercial or hatching egg production, particular attention is usually given to the protein requirement and those vitamins and minerals necessary for egg production. The nutrient requirements of other domestic or game birds, when not known, are often estimated by comparison to the most analogous domestic species.

At present there are no fixed quantitative requirements for water. There are too many factors which influence the birds' need for water to permit the establishment of firm requirements. These includes diet and physical form of the diet, inhibitors in feed ingredients, carbohydrate sources, age, breed, rate of production, environmental temperature and contaminants in feed or water systems. A handy rule of thumb is that the bird consumes 1.5 - 2. 0 times as much water as it does feed (Gupta *et al*., 2008). NRC (1984) recommended calcium and phosphorus levels of 3.50% and 0.35% for laying hens; while values recommended by NRC (1994) are 3.10% and 0.32%, respectively. In Nigeria, Ravindran (1995) obtained highest egg production from layers fed with ration containing 3.50% calcium and 0.60% phosphorus. For broiler raising in Nigeria, Oluyemi and Roberts (2000) recommended calcium and phosphorus levels of 1.3-1.6% and 0.9-1.2% for starter chicks, while for finisher chicks he recommended 1.2-1.3% and 0.8-1.0% calcium and phosphorus levels, respectively. NRC (1994) recommended the levels of 1.00% (calcium) and 0.45% (phosphorus) for starter phase and 0.80% (calcium) and 0.35% (phosphorus) for finisher phase of broiler chickens. Little information is available on vitamin requirements for poultry in the tropics and does not differ from that in the temperate areas (Oluyemi and Roberts, 2000). Nutrient requirements of broiler chicks are reviewed below herein from past works.

2.1.1 Nutrient requirements of broilers

Broilers are birds that are fast growing, ready for the market within the period 6 to 8 weeks. They are characterised by tender meat. Meat tenderness is desirable because of improved digestibility, low potential damage to teeth and low energy expenditure on cooking (Oluyemi and Roberts 2000).

2.1.1.1 Protein requirements of broilers

The dietary requirement of protein by an animal is made up of two components: the essential amino acids and the total need for nitrogen in a suitable form. Though numerous works have been done to determine the protein level that would meet the broiler chick requirement and ensure positive nitrogen balance, it would appear that there has been no concensus on the exact dietary protein level that would support maximum performance; Idufueko (1994) and Cecil, (1995) obtained adequate performance at different protein levels. Early studies by Hammond (1988), showed that protein intake of approximately 21% was close to the physiological optimum for the growing chick when the feed is fed from hatching to maturity. Manning *et al.* (2007) demonstrated that broiler diets fed at 30% protein level from day old to two weeks of age gave an initial growth stimulation that could be maintained to maturity. However, Matthew (2005) indicated that diets containing 35-40% crude protein depressed growth. Onu and Aniebo (2011) reported that the requirement for protein by young chicks up to eight weeks of age is 20% of the diet. Petterson (2007) observed optimal growth up to seventh week in chicks fed diet containing a crude protein level of 18.8%.O^{c}, showed that a higher protein level promotes a greater muscle development, while, Navid *et al*. (2010) concluded that higher protein levels promote higher feed conversion ratio and leaner carcass

On the basis of experimental observations, standards have been established to cover the needs for crude protein and its amino acid contents for poultry, under varying environmental conditions. The recommended protein level for broiler starter diets are 23% (NRC, 1994) and a lower crude protein percentage of 19% for broiler finisher diets. FAO (2006) recommended a range of 22-23% and 18-20% crude protein in the broiler starter and finisher diets respectively for tropical environment. Vahra (2002) recommended a higher protein for birds in the tropics and that protein requirements depended on the genotype and sex of the chicks, the higher protein requirement of the male than female chick has been shown by Lewis (2004). Two separate studies in Nigeria by Fetuga (1984) and Olomu (1996), suggested crude protein levels in the range of 23-24% and 19-20% for broiler starter and finisher diets in the tropics, Kruchten (2009) calculated that the protein requirement, expressed as gram per 100g gain in live weight of male chicks (1 -4 weeks of age), was about 35g protein with un-supplemented diets. The requirements were 45.3 to 50.4g protein for the 5-7 weeks period. On the other hand, Oluyemi and Roberts

(2000) estimated the protein requirement of chicks in Nigeria to be 30g per 100g weight gain. Investigating the crude protein requirements of growing chickens under Nigerian environment, Njike (2009), showed that maximum weight gain was achieved by chickens on un-supplemented diets containing 20 and 22% protein and on 18% protein diets supplemented with methiomine or methionine plus fish meal. The performance of chicks on unsupplemented 18% protein or 16% protein diet supplemented with methionine or methionine plus fish meal were comparable under tropical conditions like Nigeria.

Fetuga (1984) showed that the need for protein is essentially a need for amino acids and that the amino acid requirements of tropical livestock do not differ markedly from those of the temperate. The implication of this would be that efficient production is possible at lower protein levels, provided a proper balance of amino acids is maintained.

2.1.1.2 Amino acid Requirement of broiler chickens

The basic need of poultry for dietary protein is the supply of both essential and non-essential amino acids for their metabolism. The ability of the protein to supply these in the quantity and quality required for body synthesis is a measure of the nutritive value of protein. Baker and Han (1994), had earlier reported that excess of an individual amino acid in a diet may be harmful, as it may affect the utilisation of other amino acids. Nelson (1990) showed that the requirement for a particular sulphur amino acid is 3.5% of the dietary protein over a range of 19-27% protein. Report of Corzo *et al*. (2005) showed that lysine requirement of the chick up to two weeks of age was estimated as 1.1% of a diet containing 12.54 Mg/Kg metabolisable energy, about 13 percent less lysine than NRC (1994) recommendation.

According to NRC (1994), the Essential Amino Acids (EAA) are glutamic acid, glycine, histidine, isoleucine, leucine, arginine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. However, Payne and Lewis (2004), found that growth rate was optimal or slightly decreased between seven and twenty days of age with a diet containing 16 percent protein, 0.9 percent lysine, 0.92 percent arginine, 0.91 percent leucine, 0.56 percent valine and 0.51 percent threonine. The amount of amino acid required for chick's growth had been shown by D'Mello and Acamovic (1989) to be related to the dietary protein and increases with increase in the dietary protein. A maximum was reached around 15 percent, after which there was some

decrease in the dietary protein. This meant that a moderate deficit in all amino acids can provoke an increased consumption, as if the bird tries to compensate for the deficiency by increasing amino acid intake (Café and Waldroup 2006). Hesabi *et al.* (2008) had suggested that lysine and methionine are the most limiting amino acids in practical poultry formulations in World. Kerr *et al*. (1997) also reported that limiting amino acids could not improve growth rate of chicks by increasing lysine content over 90 percent of NRC (1994) recommendations. Fetuga (1984) recommended 1.1-1.3% lysine and 0.7-0.8 percent methionine for broiler starter, 0.95-11.1 percent lysine and 0.6-0.7 percent methionine for the finisher phase for optimal performance of chicks.

2.1.1.3 Energy requirements of broiler chickens

Birds eat in order to satisfy their energy requirements (Cheeke and Myer, 2005). At very low energy concentrations, the chick may not meet its energy requirements and at high energy concentrations, may consume less feed than is required for maximum growth and the excess energy may be deposited as fat (Donaldson *et al.,* 1985; Spring and Wilkinson, 1987). Sell *et al*. (1987) had shown that pelleting of diets and the inclusion of some oils, even in small amounts can increase the availability of metabolisable energy of the feed and optimum growth rate achieved at dietary energy concentration below the recommended levels. The ambient temperature may also influence the optimum energy intake in broiler diets, (Scott *et al.*, 1996), this is of some importance in areas with a wide temperature range. Combs (1998) indicated that broilers reared at low temperature cycle (11.7 to 18.3°C) were 0.12kg heavier than those raised at 18.3° to 35°C, but also consumed 0.68kg more feed. Scott *et al*., (1996), reported that chickens do not adjust their energy intake exactly but consume somewhat more energy as the energy concentration of the diet increases, resulting in the deposition of large amounts of fat. There was no difference observed in the daily intake between various dietary energy levels, when daily intakes were based on metabolic size (WO.75) (Lei and Slinger (1987). It is difficult to fix a basis on which dietary energy concentration can be recommended for broiler chicken production, since differences occur in response of different breeds and strains of chickens to diets with the same energy concentrations (Pond *et al*., 1995; Nowland and Pym 1998). However, Perry (2004) showed that broiler chickens fed diets with dietary energy concentrations, exhibited large differences in their growth rate, feed intake, feed conversion ratio and dressing percentage. He

also reported that birds fed high dietary energy generally required more metabolisable energy to reach the target weight than those on lower energy diets.

Donaldson *et al.* (1985) indicated that the quantity of feed eaten by poultry was inversely proportional to the concentrations of the dietary energy. Parrel *et al*. (1994) using eight diets with energy concentrations ranging from 2.3 to 3.5 kcal ME/g, observed that birds showed optimal growth to a specified live weight. The author further reported growth improvement in birds fed diets of energy concentration up to 3.3 kcal ME/g. The energy of broiler chicks fed a wide range of dietary energy (2.28 kcal ME/g to 3.61 kcal ME/g) showed no measurable differences in amount of metabolisable energy consumed per unit live-weight recorded (Nawaz *et al*., 2006), this showed that birds adjust their feed intake to meet their energy requirements. The recommended metabolisable energy density of broiler diets are 2.85 kcal ME/g (NRC 1984) and 3.20 kcal ME/g (NRC 1994). Metabolisable energy of the range 2.8 to 3.0 kcal ME per gram for optimum performance of broiler chicks raised in Nigeria had been recommended by Fetuga (1984) and Olomu (1996) this indicated that there is no standard that is adequate for all environmental conditions. It has been reported by Combs (1998) that birds eat primarily to satisfy their energy requirement and as a consequence, the caloric density of the feed therefore determines how much of a diet will be consumed, this follows that in formulating diets, the proportion of other nutrients should be fixed to the energy content, to ensure optimum performance. The realisation that energy determines intake of other nutrients has necessitated the concept of calorie-protein ratio. Halmitton (1998) and Oluyemi and Roberts (2000) suggested that inter-relationship occur between protein and energy, and that the advantage of high energy diets could only be realized by the inclusion of good quality protein in the diets. Church *et al*., (1991) reported that diets high in protein and low in energy reduced growth and efficiency of feed utilisation, but the addition of energy to these diets improved both growth and feed efficiency. On the other hand, Donaldson *et al*. (1985), observed that changes in the calorie-protein ratio of the diet had marked effects on the body composition of chicks; wider calorie-protein ratio increased the fat and gross energy content, but decreased the water content, they concluded that chicks appeared to consume relatively more energy in an effort to obtain other required nutrients presumably certain amino acids, and much of the additional energy consumed was deposited as fat in place of water in the carcass. Fasuyi and Aletor (2005) reported that calorie-protein ratio is an important factor in growth and energy utilisation. Durrani *et al*. (2006)

reported that the higher the protein content at each energy level, the greater was the growth rate. Combs (1998) pointed out that the relationship between energy and all essential nutrients must be constant and this is particularly important in order to maintain amino acid balance. According to the author, the calorie-protein ratio is mathematically the addition of metabolisable and productive energy derived from carbohydrates, fats and proteins divided by the percent crude protein. Furthermore, he recommended a calorie-protein ratio ranges from 132 - 155 in the starter and finisher diets. Glick (1981) suggested a calorie-protein ratio of 92.4 - 94.6 and 107.8 -110 productive energy in starter and finisher broiler diets respectively .These findings are in contrast to 99, recommended by the NRC (1984). Panda and Rao (1993) fed four levels of protein namely 15, 18, 21 and 24% and a fixed energy level of 3.0 kcal ME/g in the diets fed to three breeds of broilers. The optimum calorie-protein for starter broiler was 125-142, with a protein content of 21 - 24 percent. In another study (Panda and Rao, 1993), the effect of three levels of protein namely, 18, 22 and 26% with a constant energy: protein ratio of 130:1 was studied in starter broilers, they concluded that 22 percent with calorie-protein ratio of 130:1 was optimum. Knowles *et al*. (1998), reported that rearing chicks on commercial diets containing energy-protein ratio slightly above or below the recommended optimum level of 145, did not affect growth rate, and they noted that energy-protein ratio of 145 with 20 percent protein gave the best performance. Olomu (1996), using different protein levels, 17, 20, 23 and 26% and energy levels 2.8, 3.0 and 3.2 kcal ME/g on broilers, reported that maximum weight gains and efficiency of feed conversion were obtained on diets containing 23 and 26% protein levels. As the dietary energy level increased, there was a slight depression in weight gain; the best performance of chicks being obtained on the diets containing 2.8 kcal ME/g. Oluyemi and Roberts (2000) considered the mutual relationship between dietary energy and dietary protein of the fowl. They pointed out that the calorie and crude protein may individually be adequate, but may be poorly adjusted to the extent that this reduces the performance of the birds or causes the deposition of fat in their carcasses. They concluded that broiler requires more protein and energy than layers, to cope with the relatively higher rate of physiological activities .The calorie-protein requirement is affected by the quality of the protein, the environmental condition, and the rate of growth of the chicks. It is obvious that the different classes of nutrients in the diets cannot be treated in isolation.

The amounts of protein, essential amino acids, vitamins and minerals, must be adjusted in a fixed ratio with the energy to influence protein and energy requirement of laying birds and the temperature used will be above or below the 24°C requirement levels. Reid *et al*. (1984) reported increased egg production in single-comb White Leghorn pullets fed 16% protein *as* the metabolisable energy of the diet **i**ncreased from 2.42 through 2.64 and 2.68 to 3.08 kcal ME/g diet. The study futher suggested a 2.25 percent increased in egg production for each 0.22 kcal ME/g diet. Finding was confirmed by Patrick (1996), who reported that there was a linear relationship between energy value of a diet and efficiency of egg production in White Leghorn hen.

2.2 Soyabean composition and Utilisation

The main soyabean growing areas in Nigeria are in the southern Guinea savanna zone, where a rainy season of five months is discouraging the cultivation of groundnut (Ashaye and Balph 1985). Benue State is the main soyabean growing area, followed by Abuja in the Federal Capital territory and the southern part of Kaduna State. The total soyabean production in Nigeria has been very erratic. At present, there are few incentives for farmers to produce soyabean. Prior to 1975, the market value of soyabean was ₦66 per tonne, in 1975/76, was ₦99 per tonne, in 1976/77, ₦130 per tonne and in 1981/82, ₦155 per tone and soyabean meal in selling for about ₦50 per kilogram in 2009 and ₦120 per kilogram in 2011 (Ogbe and Affiku 2012).

2.2.1 Nutrient composition of soyabean

The soyabean seed contains proteins, carbohydrates, vitamins and minerals. The protein and lipids are the principal nutrients of importance, accounting for about 60% of the total nutrient content, but about one third also consists of carbohydrates including the polysaccharides and water soluble carbohydrate fractions (Dabrowsky *et al.*, 1989). Normally, soyabean are processed to obtain their optimum nutritional value for food or feed. Oyenuga (1968) reported that soyabean grown in the country is rich in protein (44.08%), good in its content of oil (19.10%) and has an average amount of fibre and ash; 5.71 and 5% respectively. Ologhobo (1980) gave the proximate composition of two types of soyabean as follows; GE 109: 36.69% crude protein, 21.59% ether extract, 3.85% crude fibre, 8.24% ash and GE 104: 36.66% crude

protein, 26.62% ether extract, 4.69% crude fibre, 7.48% ash. However, Balloun (1980) reported values of 44-49% crude protein, 20% ether extract, and 2.8 to 6% crude fibre,

The proximate analysis reported by Tewe (1984) for whole undefatted soyabean showed the following, 38.0% crude protein, 18.0% ether extract 5.0% crude fibre and 5.0% ash. Waldroup *et al*., (2004) indicated that heated soyabean or full-fat soyabean contain high quality protein (38-42%), as well as a rich source of energy, due to its oil content of about 18-22%. Elboushy and Roodbeen (1980) contended that the nutritional defect in raw soyabean resulted from the unavailability of amino acids from poor caloric utilization. Hendricks *et al*. (1990) indicated that the limiting amino acids of soy-protein relative to chick requirement are sulphur amino acids, threonine, valine and lysine, in that order. Kerr *et al*. (1999) showed that there are differences in the amino acid content and availability among the different varieties of soyabean, but the availability of the different amino acid were still quite high. The amino acids of soyabean meal have been shown to be highly available. Kerr *et al*. (1999) obtained availability ranging from 92.0 to 100%, for a number of acids in several soyabean meal samples; lysine availability ranged from 93.5 to 99.9% and methionine from 97.3 to 99.3%. Liener (2005) reviewed the nutritional value of soyabean proteins in the whole bean and in various processed fractions, including the immature grain and beans, meal and flour. The author concluded that the liminting amino acids on the basis of chemical score are the sulphur containing methionine and cystine and the most valuable attributes of soy-protein is the fact that it contains more lysine than most plant proteins. It has been shown that the amino acid content in terms of gram per gram total nitrogen, for isoleucine is 4.5 and 4.8 in the seed and cake, respectively, 7.8 of leucine in both seed and cake 6.4 and 6.1 of lysine in seed and cake, respectively. Similar patterns were also recorded for phenylalanine, alanine, aspartic acid, glutamic acid, proline and serine, in both seed and cake of soyabean. Higher availability of amino with an average of 97.3% was reported by Vander Klis *et al*. 1997). Soyabean has the most complete amino acid profile of all the several vegetable proteins except, that it is deficient in methionine (Ologhobo, 1980). However, Krogdhal *et al*. (1995) reported that this deficiency was due to non-availability rather than to absence. Orthoafer (1998) indicated that the amino acid availability in soyabean meal is about 91%. Balloun (1998) stated that soyabean meal has all known nutritionally essential amino acid, except that it is deficient in sulphur amino acids, methionine and cystine. It has been reported by Oyenuga (1968), that climate and temperature rather than soil and varieties, tend to cause variations in oils

of soyabean. The oil belongs to the linoleic acid group, which is an essential fatty acid required for poultry and for laying hens in particular. The work of Carew and Nesheim (1993) demonstrated that the growth simulation in chick resulting from flaking of heated soyabean was due to increase absorbability of the oil and as a consequence, increased metabolisable energy. Carew and Nesheim (1993) showed that pelleting of the entire diet containing heated soyabean resulted in increased availability of the oil to the chick. Palissero and Sumpter (1992) reported that oil in cooked full-fat soyabean created no adverse effect in fish diets, but causes soft pork in pigs. Soyabean 16 to 21% of oil and the oil content vary with and climatic environment during growth, Ologhobo (1980) reported that the soyabean fatty profile included traces of lauric acids (0,083%) in GE palmitoleic acid, with 0.68% in GE104 and 0.137% in no traces of myristoleic and linoleic acid were in any of the two soyabean varieties. However, an abundance of linoleic acid was present with GE104 and GE109 containing 48.83% and 55.33% respectively, oleic acid ranged 27.32% GE104 to 23.43% of GE109, palmitic acid was (1.04%) in GE 109 and 9.705% in GE104.

The importance of minerals as chemical constituents and their functions in the body cannot be over emphasised, subsequently, soyabean has been shown to be a good source of number of minerals compared to grains in practical poultry (Balloun, 1998). Balloun, (1998) also indicated that when soyabean replaces animal protein in the diet, several minerals be supplemented, particularly calcium and phosphorus. Ologhobo (1980) reported some values of the major and minor minerals in soyabean meal, which were in close agreement with the values of 220mg/100g of calcium, 586mg/100g phoshorus and 7.0mg/100g iron, given by Oyenuga (1968). Various vitamins are available in soyabean meal (NRC 1994).

Soyabean meal has been shown to furnish about 30% of poultry or chicks starter requirements for riboflavin, niacin, pantothenic, thiamine, folic acid, choline and its fairy rich in carotene (Nwokolo and Bragg, 1996). It has been shown that soyabean meal offers some resistance to ascarid infection in chickens. Latshaw and Clayton (1996) demonstrated that a batch diet supplemented with14.2% of soyabean meal and skim milk every second day increased the resistance of young chicken to the parasite *Ascaridia galli* as judged by the comparative number of worms remaining in the chicken at autopsy. Carew and Nesheim (1993) reported that dehulled soyabean meal has high energy content, metabolisable energy content of dehulled soyabean meal

(50% protein) was given as 253 kcal ME/g. They also concluded that the metabolisable energy content increased as the fat and protein content increased, with decreased crude fibre. Hill and Renner (1993) reported that heated soyabean had more energy (3.36 to 3.70 kcal ME/g), compared with 2.84 kcal ME/g for heated ground soyabean. Scott (1996) observed a metabolisable energy of 2.51 kcal ME/g for chicks and 2.52 kcal ME/g for laying hens for a 46.4% crude protein soyabean meal,

Wiseman (1998) presented data that showed differences in the metabolisable energy content of full-fat soyabean passed by different methods; extruded soyabean had higher metabolisable energy content (4.28 Kcal ME/g), than toasted (3.73 kcal ME/g), micronized (3.68 kcal ME/g), jet- sploded (3.52 kcal ME\g) or raw soyabean (3.23 kcal ME\g).

2.2.2 Anti-nutritional factors of soyabean

Soyabean like most legumes contains some biologically active substances in their raw state. The substances are generally referred to as 'biologically active substances' or anti-nutritional factors. Some of these compounds are toxic while others inhibit the availability of desired elements and substances that are otherwise useful to various animals. These anti-nutritional factors can also inhibit growth, reduce fat absorption, decrease metabolisable energy of diet, enlargement of the pancreas, stimulate hyper secretion pancreas, and cause agglutination the red blood cells (Ahn 1990). Trypsin inhibitor, a globulin, was one of the first soyabean components processed as an anti nutritional factor, following its discovery (Bowman, 1944), trypsin inhibitor effect accentuating marginal of methionine, cystine and other amino acids. Almquist and Merrit (1953) isolated another anti-nutritional factor from raw soyabean - phytohemagglutinin, which was chemically shown to be an albumin. Liener (2005) showed hemagglutinin to agglutinate red blood cells *in vitro*.

However, because it is readily inactivated by pepsin during digestion, it is doubtful that ingested hemagglutinin can agglutinate red blood cell *in vivo*. Oyenuga (1968) suggested that trypsin inhibitor might produce growth retarding effects through increased stimulation of small intestine of young chicks and resulted in enlarged due to exceptionally high concentration of trypsinogen. Ologhobo (1980) have indicated that when birds grow older they become less sensitive to the toxic effects of raw soyabean. Donalson *et al*. (1985) observed that the growth rate and feed

efficiency were negatively affected by amounts of raw soyabean meal when used in the diet at different levels. Carew and Nesheim (1993) showed that the higher levels supported better growth of animal. Summers and Leeson (1996) have observed that the performance of layers fed raw whole soyabean equaled that of hens fed diets containing processed soyabean meal. Gestetner (1996) reported that saponins pass through the entire gastrointestinal tract of chicks without being hydrolysed or absorbed.

A review by Patrick (1996) revealed that for the first 8 to 12 weeks of life, chicks showed growth depression on a raw soyabean diets, but eventually gained weight at a rate equal to these on heated soyabean diet. Ologhobo *et al.* (2006) reported that destruction of trypsin inhibitor activity and urease activity in raw soyabean meal was a function of cooling time and the moisture level of raw soyabean.

2.2.3 Processing of soyabean

It has long been recognised that the nutritive value of soyabean markedly improved by heating. Heating conditions is required for satisfactory utilisation of raw soyabean by poultry. The major criteria used to evaluate the effects of treatment are the growth rate of monogastric animals and the rate and extent of protein digestion *in vivo* and *in vitro*. Early studies of Oyenuga (1968) showed that cooking greatly improved the nutritional value of soyabean. Since, the majority of the anti-nutritional factors are heat labile. Renner and Hill (1990) showed that heat treatment which produced maximal rate and efficiency of chick growth also produced maximal utilisation as measured by metabolisable energy value. Gestetner *et al*. (1990) obtained evidence that certain amount of heat was required for maximum liability of methionine and cystine, they also suggested that the availability of the amino acids in the processed meal was higher than that of the raw soyabean meal. However, Patrick (1996) showed that regardless of moisture, over 95% of trypsin inhibitor content of the raw soyabean meal is destroyed within 15 minutes.

Most, if not all of the anti-nutritional activities in raw soyabean are destroyed by moist heat. However, 10-15 minutes of moist heat treatment at 100^0C inactivates these inhibitors Renner (1990). The effects produced by heat were evaluated by Ologhobo (1980), he observed that the removal of the inhibitor increased the protein efficiency ratio, and a greater increase in the digestibility of the protein Hill and Renner (1988) reported that severe heat treatment reduced the

digestibility and availability of methionine by about half, while the availability of lysine was reduced to one third in over-heated soyabean proteins. These workers concluded that the "unavailable" peptides and more amino acids were recovered in the ileum of chicks. Kawamura (1989) suggested that temperature applied, the time of exposure and the presence or absence of moisture were the factors in heat treatment that influenced the digestibility of soyabean meal proteins. He also stated that the destruction of the inhibitor and the improvement in the nutritive value was paralleled by the enzymatic liberation of available cystine as measured *in vitro*, the protein efficiency ratio increased at same time.

2.2.4 Preparation of defatted soyabean meal

Soyabean is processed to obtain their optimum nutritive value for both food and feeds. Laboratory methods of processing bear little resemblance to the sophisticated equipments used in large scale soyabean processing. Commercial processing of soyabean usually involves the removal of as much oil as possible with a view of producing meals with good qualities (Renner 1990) Moist heat treatment inactivates the anti-nutritional factors present and makes the flour and meal more suitable for animal and human consumption. Dehulling is usually practiced after cracking to reduce fibre content, by passing the cracked soyabean through dehuller, equipped with shakers and aspirators, dehulling is usually carried out to improve efficiency of the extraction plant, since the hulls contain very little oil. The dehulled, cracked soyabean are conditioned to about 10-11% moisture, at 63-74°C and then flaked by passing through smooth rolls in flaking mills. Strong springs are used to exert proper pressure on the rolls to ensure relatively thin flakes of uniform thickness. Flaking ruptures the cells in the soyabean and reduces the distance that oil and solvent must diffuse, thereby facilitating extraction with organic solvent. In direct solvent extraction, the choice of solvents in the extraction of soyabean for oil and meal is based primarily on the ease with which the oil is removed from the flakes.

Hexane (boiling point 66-69°C) is used in the United States, heptane (boiling point 89-98°C) is sometimes used in climates where outside temperatures are high in order to reduce explosion hazards. Cyclohexane (boiling point 71-85°C), ethanol and trichloroethylene have been used commercially. The soyabean flakes flow by conveyor to the extractor, with the pumping of solvent and miscelle in a progressive step wise counter current flow to flakes. Solvent and most of the oil is removed, which is latter processed to edible oil via degumming, alkali-refining,

deodourisation, and hydrogenation. For feed use, the hexane-laden flakes are passed through a desolventizer-toaster, which removes the hexane and simultaneously toast the flakes to obtain optimum nutritive value of 44-45% crude protein and about 1% residual oil or fat, After drying and cooling, the flakes are ground into meal (Tewe 1984).

Other processing methods, apart from solvent-extraction method, includes hydraulic press, screw-press and pre-press solvent extraction .The hydraulic press involves crushing the seed into flakes, cook the flakes thoroughly in steam, the resultant mass is formed into cakes, these are placed in hydraulic press system, where as much oil as possible is pressed out and the meal is left. The meal often contains about 4-5% fat or oil and 38-40% crude protein .The screw-press (expeller method), is similar to hydraulic press but the cooked flake is passed through a screw expeller which releases the oil. The resultant meal has about the same protein and fat content as that of the hydraulic press.

Processing of full-fat soyabean is necessary to achieve the optimum nutritive value. In addition, overcoming the anti-nutritive factors naturally occurring in raw soyabean, processing in necessary to optimise the energy value of soyabean (Patrick 1996; Wiseman, 1998). Several methods of processing whole soyabean are available. Most of these systems that have been developed for commercial or on-the-farm use fall into three basic methods. The first method is a simple, easily applicable processing method of low technology. It involves boiling or steam-cooling of whole raw soyabean (NAPRI, 1984). The nutritive value of the end product depends greatly on the temperature and time of boiling, generally boiling for 30 minutes at 100°C, has been shown to support improved performance in birds, without loss of intrinsic nutrients. This method is important for on-the-farm and small scale processing of full-fat soyabean. Sun-drying is employed, before grinding into meal, for mixing into the diet.

The second method uses dry heat. In these systems, the raw whole soyabean is subjected to a gas flame or other heat source for brief intervals to accomplish the cooking .The process does not disrupt the cells and the soyabean must be ground, rolled and flaked before mixing into the diet. These methods includes; Toasting/Roasting (Raghavan 1988), Microwave systems (Hafez *et al*., 1989), Micronising (Gestetner *et al*., 1990) and Jet-Sploding (Morill, 1991). In toasting and roasting dry heat is used to toast or roast the raw whole soyabean. Apparently, high moisture content during toasting facilitates destruction of the trypsin inhibitor in less time and with less

heat, and before other essential nutritional factors are destroyed thus rendering them unavailable to the bird (Bornstein and Lipstein, 1995).

A recent development is the roasting of full-fat soyabean in a heated salt bed. By adjusting salt bed temperature and residence time of the raw soyabean, proper cooking can be obtained. This process has been used successfully to produce a full-fat soya flour for human use. Microwave systems can be used to cook raw soyabean, for example infra-red cooking. In this method, soyabean are exposed to the microwave at short intervals to ensure proper cooking. Broiler has been reported to grow well on microwave-cooked soyabean with comparable feed conversions and pancreas weight, body availability, costs, handling and equipment. Full-fat soyabean can be a great source of energy, especially when higher energy feed that are desirable. Full-fat soyabean can as well provide linoleic acid, which is an essential fatty acid required for poultry in general and for laying hens in particular. Carew and Nesheim (1993) indicated that the oil present in full-fat soyabean is not freely available for digestion and absorption, unless the cells in which it is contained are rupture, this can be attained by the several methods of processing full-fat soyabean, though the nutritive value of the final product will reflect to some extent the variations among these methods.

Other advantages that have been attributed to the full-fat soyabean include the fact that is a granular material that can be handled at a lower cost than fat in the feed mixing operation. Also fat imclusion within the matrix of feed particle, rather than sprayed on the surface permit feeds with a higher fat content to be made into satisfactory pellets. Additionally, the quality of the fat is higher than that obtained from most sources of added fat (Balloun 1998). Essentially, the combination of hydraulic press/screw-press with solvent extraction process, that is, the cooked soyabean is first pressed for removal of most of the oil while the resultant meal is subjected to solvent extraction process, for the removal of more oil (Parrel, 1994).

2.2.5 Preparation of full-fat soyabean

A relatively new development in the use of soyabean is the feeding of "cooked" full-fat soyabean. There is now a general agreement that processing of full-fat soyabean produces valuable product that can be effectively utilised in poultry feeds in many parts of the world. The interest in using the product on a much larger scale in commercial feeds in increasing (Horani

and Daghir1997) In certain parts of the world, this interest has been greater due to the fact that full-fat soyabean are not only an excellent source of protein, but, they are an invaluable sources of energy as well. Full-fat soyabean contains approximately equal amounts of energy as corn (the highest energy source among cereals, used in poultry feeds) however, its protein content is more than four times that of corn (Waldroup, 1985). Thus, it is apparent that in places where the use of added fat in poultry diets is limited due to factors such as weight gain were reduced compared to defatted soyabean meal (Hafez *et al*., 1989). Micronisation involves short term exposure to radiant heat at high temperature. Micronised whole soyabean fed to broilers had reduced nutrient digestibility (Combs 1998), he noted that the urease value of the beans were very low, possibly indicating over-heating.

The Jet-sploder process involves subjecting full-fat soyabean to super-heated air for the desired period of time, followed by passage through a roller mill. Whole full-fat soyabean are fed by gravity into heat exchanger, air heated at about 315°C is pumped through jets into the exchanger, quickly heating the soyabean and also transporting them through the unit. After the desired time in the heat exchanger, the soyabean are fed through rolls. The temperature of the beans, as well as, the pressure developed by the super-heated moisture and the roll are responsible for the changes that occur .There are several advantages of this type of system, since moisture is not added in the process, it is not necessary to dry the processed products. Also most of the air is recycled, therefore, the use of energy for providing heat is very efficient. The short time used for heating the soyabean means that high production rate is possible. Proper processing conditions for the raw soyabean and the type of livestock fed, however results to date suggested that soyabean should be processed for just over one minute and exit temperature of about 170% (Morill, 1991). Njike *et al*. (2009) clearly demonstrated that autoclaving the ether extracted soyabean meal at 121°C for 15-20 minutes was the best method for producing adequately heat-treated soyabean meal.

The third category is the Extrusion-cooking process of particular interest is the extrusion-cooking process in the production of full-fat extruded meal. SEEPC Nigeria Limited, Lagos and International Institute of Tropical Agriculture (IITA), Ibadan, are currently involved in the processing of locally produced soyabean into full-fat extruded soyabean meal. This is to be incorporated into diets for different classes of poultry and pigs. The cooking process is preceded

by dry cleaning of the raw soyabean, employing normal feed-milling equipment, containing a magnet the raw soyabean will be ground in a hammer mill, prior to extrusion-cooking. The ground soyabean should be stored for a s short a period as possible, so that rancidity formation is minimised, When cooking full-fat soyabean via extrusion process main objectives sought are; firstly control of the anti-nutritional factors which are contained within the raw soyabean, particularly trypsin, inhibitor. Secondly, once, the hulls of the soyabean have been cracked, ground, the soyabean have a tendency towards .rancidity, within a short period of time, caused by the fat-splitting enzymes present in the soyabean oil, Inactivation of the fat splitting enzymes, lipases and lipoxidase, will help maximise the storage period of the extruded product .Thirdly, the oil which is contained within the soyabean is surrounded by small sacs, that reduces its energy availability, these sacs are ruptured by the pressure and friction present in extrusion process. The last objective is at counter purpose with the above objectives, that is, while cooking products must be taken into consideration, that a minimum heat damage is done to the proteins; since over-cooking renders the product less biologically suitable due to vitamin and protein damage (Ologhobo *et al.,* 2006).

A properly designed and energy efficient extrusion cooker utilises moisture in the form of water, steam or a combination of the two during cooking. The main elements of the machine are, the Live-Bin Discharger, the Pre conditioner and the Extruder section itself .The ground soyabean is fed to the live holding bin above the extruder and conveyed to the extruder barrel by the variable retention pre-conditioning chamber. The pre-conditioning chamber is operated at atmospheric pressure and low pressure steam is injected therein, from the pre conditioner, the product is dropped into the extruder barrel where additional moisture in form of water or steam can be added and heat from friction and externally applied steam are used to cook (pressure-cooking) the soyabean to inactive trypsin inhibitor and reduce the urease activity (Tewe 1984).

The resulting product is a granular material that does not need additional grinding, there is usually considerable amount of cell rupture and the nutrients are highly digestible. Product moisture after discharge from the final die to the extruder is generally in the range between 12 - 14%. Post-extrusion cooling of the product in a horizontal tray type cooler or a rotating thumbling type cooler, this will lower the moisture content by 2 to 3% i.e. 10 to 12% final moisture content. This will render the product suitable for storage in holding bins, sacks or other

storage devices .The process described above is for the Moist Extrusion cooking of full-fat soyabean, Dry Extrusion Cooking is also possible, the only condition is that, the full-fat soyabean to be extruded must have high moisture content about 13 to 14% and above, hence water or steam addition is not necessary and the soyabean can be extruded.

To ensure minimum protein damage during the heating process, short time/high temperature is considered best, this can be compared to the flash pasteurization of milk that is utilized in the dairy industry. Summarily, when cooking full-fat soyabean via Extrusion process, one must consider control of growth inhibitors, control of enzymes responsible for rancidity, rupturing of the oil sacs and minimisation of protein damage during cooking .When compared to other cooking methods, it has proven to be effective from a cost and energy stand point.. A more recent alternative towards minimising the effect of sky-rocketing prices of grains, particularly, maize is the production of extruded cereal/soyabean meal mixtures, such as extruded corn-soy meal, extruded sweet potato-soy meal, extruded cassava - soy meal, in varying proportions, such as 70:30, 50:50 or 60:40. In case of extruded corn-soy meal, you can either use a whole corn or degermed corn in combination with dehulled or undehulled full-fat soyabean, at different ratios. When these cereal-full fat soyabean meal mixtures are used in poultry feed, supplementation with lysine and/or methionine is necessary, though it should be noted that most cereals are high in methionine, while full-fat soyabean is high in lysine (Sell *et al*., 1987).

2.2.6 Soyabean utilisation in broiler rations

Kahn *et al*. (1988) indicated that feeding of raw soyabean causes the gall bladder to contract, increases excretion of bile acids, lower intestinal proteolytic activity in chicks and affect methionine oxidation in rats. The most pronounced effects in chicks appear to be the decreases in protein and fat digestibilities, whereas in rats, these factors are less important. Han *et al*. (1998) observed that chick growth was retarded by unheated soyabean meal in the diet, even though adequate amounts of high quality protein were added from other sources. The body weight data of Latshaw and Clayton (1996) indicated that each increment of raw soyabean meal resulted in a further depression of chick's growth.

The metabolisable energy and nitrogen utilisation also showed a step wise decrease of chick growth. However Morill (1991) stated that protein digestibility and absorption of heated

soyabean was significantly higher than that of raw or over-heated soyabean. Likewise, Nesheim and Garlich (1996) showed that the protein in diets containing unheated soyabean meal was only 54% digested compared to 85% for heated meal. Chicks fed raw soyabean meal suffer from amino acid deficiency due to poor biological value of protein in raw soyabean meal, this was given as the major reason for poor utilisation of unheated soyabean meal by chickens. Tewe (1984) stated that when small amounts of raw soyabean meal are included in diet for chicks, growth rate was depressed, the pancreas increased in size, absorption of fat was decreased in young chicks up to 2 to 3 weeks of age and metabolisable energy of the non-fat portion of the diet was reduced. Bornstein and Lipstein (1995) reported that the susceptibility of chicks to the negative effects of under-processed soyabean meal decreases with age. He also showed that the addition of animal protein improved the diets for chicks, irrespective of the type of soyabean meal used. Summer and Leeson (2006), working on the utilisation by chicks of differently heat treated commercial soyabean meal (subnormal, normal and over-heated), revealed that mean body weight, feed intake and feed coversion did not differ significantly among chicks fed a practical diet containing any of the three heat treated soyabean meal. Also, the performance and relative pancreas weights of chicks fed subnormal processed soyabean meal were comparable to the other dietary treatments.

These results suggested that cooking temperature used by most soyabean industries, even though lower than those recommended by laboratory studies, were sufficient to destroy growth inhibiting factors and factors responsible for pancreatic hyper trophy.

Latshaw and Clayton (1996) evaluated full-fat soyabean in broiler diets using soyabean that have been cooked in a rendering plant cooker and in an autoclave to examine the effects of various cooking times and moisture levels. Gains of chicks fed properly cooked soyabean were equal to those of chicks fed the standard commercial diets. Combs (1998) reported that broilers fed cooked whole soyabean grew almost a well as did those fed diets with solvent extracted soyabean meal. Kahn (1988) conducted feeding trials with chickens using soyabean that had been cooked in several ways and they did not get quite as good gains on the cooked soyabean as on the soyabean meal diets and suggested that flaking the soyabean might be necessary to obtain optimal utilisation.

Carew and Nesheim (1993) demonstrated conclusively that the oil in unheated ground soyabean was not as well absorbed as the oil in heated soyabean flakes. They suggested that a greater cell disruption occurred in the flaking process which released a greater amount of oil. Carew and Nesheim (1993) observed that chick gains and fat absorbability were significantly improved by pelleting diets containing cooked ground soyabean. Hill and Renner (1996) observed that uncooked and poorly-cooked soyabean had less digestible fat and lower metabolisable energy values than properly cooked soyabean. Unheated flakes also depressed the digestibility of added soyabean oil, due to adverse effects on the pancreas. Han *et al*. (1998) have also demonstrated the improvement in chick performance and in fat digestion that could be obtained when diets with roasted soyabean were pelleted. Although good results were obtained, when properly cooked soyabean were ground, performance was markedly improved when diets were pelleted which aids in increased cell rupture. Kahn *et a*l. (1988) conducted broiler feeding trials with infra-red cooked and expanded whole soyabean and found that it was possible to process unextracted soyabean in such a manner to obtain a nutritive value similar to that of toasted soyabean meal plus soyabean oil. Balloun *et al.* (1998), Hill and Renner (1996), demonstrated that extruded and infra-red cooked soyabean could be satisfactorily used in broiler diets. It was observed in these studies in which full-fat soyabean product completely replaced the solvent extracted soyabean meal, that pelleting of the diet, appeared necessary for maximum performance;, possibly as a result of reduction in bulkness of the diets, that is typical of extruded soyabean.

Waldroup *et al*. (2004) fed soyabean meal with added oil, infra-red and extruded soyabean meals to chicks, all diets were isocaloric (3.12 kcal ME/g) and isonitrogenous 23% crude protein). He observed that diets containing extruded soyabean meal supported equal or superior performance to that resulting from soyabean meal with added soyabean oil and infra-red soyabean meal. Balloun (1998) conducted, feeding trials with broiler chicks using unextracted, extruded and infra-red cooked soyabean; in general, extruded soyabean were superior to infra-red cooked soyabean and were equal to 49% soyabean meal, when the energy of the diets were equalized. Renner (1996) and Nesheim (1996) processed whole soyabean in a still-air oven and were able to obtain results comparable to solvent extracted soyabean meal in chick feeding trials.

Studies of Morill (1991) demonstrated that fine grinding did not result in the same degree of cell rupture as did pelleting, shown by weight gain, feed efficiency and nutrient digestibilities. Waldroup and Hazen (1985) determined the maximum amount of various types of full-fat soyabean that could be incorporated into all-mash broiler feeds without reducing performance. They concluded that not more than 25% full-fat soyabean cooked by dry roasting, or extrusion process with or without steam, should be included in all-mash broiler diets. Lei and Slinger (1987) examined the fatty acid composition of the broilers fed diets containing extruded whole soyabean. The fatty acid composition of the broilers reflected that of the dietary fat; birds fed the diets with extruded soyabean had higher levels of unsaturated fatty acids that are characteristic of soyabean oil. Hafez *et al*. (1993) were able to replace soyabean meal with extruded soyabean in broiler diets with no adverse effects on body weight, feed coversion and mortality even though the diets were not pelleted. Pancreas weight were slightly increased but abdominal fat was decreased by feeding the extruded soyabean. Carew and Nesheim (1993) indicated that the method of extrusion, dry or wet extrusion of soyabean had no effect on performance of chicks, but that supplementation of diets with methionine resulted in greater increased weight gain and gain-to-feed ratio for chicks fed extruded soyabean than chicks fed the control extracted soyabean meal diet. Testing a batch of micronised full-fat soyabean at various levels of inclusion in broiler feeds, with graded levels of metabolisable energy content; Renner (1990) showed that micronized full-fat soyabean gave acceptable performance even at high level of inclusion in starter mash, while in finisher mash, it gave comparable performance to extracted soyabean meal, when it was less than 20% of the diet, growth was depressed significantly at level of 22% and 33%. The weekly average body weight of chicks on cooked whole soyabean diets was significantly higher than those on groundnut cake, indicating some influence of the protein content and quality of cooked whole soyabean (Parrel, 1994).

Olateru-Olagbegi (1994) showed that diets containing cooked soyabean were highly consumed and consequently led to weight grains, because of the high palatability and balanced amino acid profile. Sell *et al*. (1987) observed that broilers fed diets in which a part or all of the extracted soyabean meal was replaced by extruded soyabean, had body weights equal to chicks feed extracted soyabean meal and had superior fed utilisation. Ologhobo *et al*. (2006) reported that 100% inclusion in broiler pelleted feeds containing full-fat soyabean processed by three different methods (dry extrusion, roasting and dry-heating followed by rolling) gave similar performance

compared to a commercial diet containing regular soyabean meal. Nig and Chen (2002) reported the results of a few field trials with processed full-fat soyabean, these trials showed better response to this product, when the level of inclusion was limited to a maximum of 29%. Gad (2007) carried out work to assess broiler performance as affected by the degree of processing micronised or extruded full-fat soyabean. The results showed that there were no significant differences, due to degree of processing, in broiler weight gain or feed conversion. No major health hazards to the hen have been noted from inclusion of heat-treated soyabean Mortality rate, liver fat, body weights and other indicators of metabolic and physiological activities all support the view that properly processed full-fat soyabean can be use as alternative protein source for broiler diets, if economically justified Liener (2005).

2.3 *Moringa oleifera* Plant in Poultry Nutrition

Plants generally contain chemical compounds (such as saponins, tannins, oxalates, phytates, trypsin inhibitors and cyanogenic glycosides) known as secondary metabolites, which are biologically active (Soetan and Oyewale, 2009). Secondary metabolites may be applied in nutrition and as pharmacologically – active agents (Soetan and Oyewale, 2009). Plants are also known to have high amounts of essential nutrients, vitamins, minerals and fatty acids and fibre (Gafar *et al*., 2012). Plant oils obtained from seeds and leaves such as *Moringa oleifera* are in high demand for their medicinal value. Apart from the medicinal uses, *Moringa oleifera* was reported to be a good source of vitamins and amino acids (Olugbemi *et al*., 2010). *Moringa oleifera* was claimed to boost the immune systems (Jayavardhanan *et al*., 1994; Olugbemi *et al*., 2010). The leaves and green fresh pods of *Moringa oleifera* are used as vegetables by human and are rich in carotene and ascorbic acid (Vitamin C) with a good profile of amino acids (Makkar and Becker, 1999). *M. oleifera* is also used in livestock feeds and the twigs are reported to be very palatable in ruminant nutrition (Sarwatt *et al.*, 2002; Kimoro, 2002; Kakengi, *et al.*, 2007). The edible leaves are very nutritious and are consumed in Nigeria, and also, its inclusion into layers diets induced double yolks, Akangbe and Abu 2011, Aremu *et al.*, 2012 The *Moringa* seed oil is reported to contain 80.4% polyunsaturated fatty acids (Anwar and Rashid 2007; Ogbunugafor *et al*., 2011). *Moringa oleifera* seeds extract was reported to have antibacterial properties and conclusion was made to investigate it as a phytotherapeutic agent to combat infectious agent (Patel, 2011). Most parts of the plant have been ued in folk medicine in Africa

and south Asia (Fahey, 2005). The medicinal effects of the plant were ascribed to their possession of anti-oxidants, which are known to suppress formation of Reactive Oxygen Species (ROS) and free radicals (Sofidiya *et al*., 2006; Ogbunugafor *et al.*, 2011, Ogbe *et al*., 2012).

In developing countries (like Nigeria), sources of animal's drinking water may be contaminated with suspended materials and bacteria but unknown to the animal owner(s). In the human, each year, millions of children are known to have died in developing countries as a result of infections caused by consumption of unclean water (Jose *et al*., 2010). *Moringa oleifera* leaves and seeds are said to be very good and safe for water treatment; as synthetic chemical compounds (alum) may be carcinogenic (Sarwatt et *al.*, 2002b). Plant substances that are foods are of little or no side effects. About 25% of the prescribed medicines today are substances derived from plants (Ngaski, 2006).

Table 1: Proximate Composition of *Moringa oleifera* Seed and Full-fat Soyabean

Parameter (%)	***Moringa oleifera* seed**	**Full-fat soyabean**
Crude protein	35.90	36.00
Crude fibre	9.00	6.00
Ether extract	16.20	18.30
Ash	12.00	9.00
Dry matter	95.30	92.50
Sources:	Makkar and Becker (1997)	Ologhobo *et al*. (2006)

2.3.2 Mode of Action of *Moringa oleifera*

Antimicrobial and antioxidant effects of *Moringa oleifera* seeds have been discussed by some researchers. Jabeen *et al*. (1999) reported that the antimicrobial properties of the *Moringa oleifera* seed extracts may be due to the presence of lipophilic compounds. These compounds may attach to the cytoplasmic membrane. The authors also suggested that extracts of *Moringa oleifera* seeds may contain antibiotic metabolites, such as carboxylic acid, 2,4-diacetyl phloroglucinol, and cell wall-degrading enzymes and chitinases. The antioxidant effect of *Moringa oleifera* seed extract and fruit was explained by Lugman *et al.* (2010), who noticed that it was due to the presence of polyphenols, tannins, anthocyanin, glycosides, and thiocarbamates, which remove free radicals, activate antioxidant enzymes, and inhibit oxidases.

2.3.3 Chemical Composition of *Moringa oleifera* Seed

The protein content of seeds does not vary substantially from place to place. There is a need to make proper use of the large amount of the residual seeds left after the extraction of growth promoting component. With the aim of using the extracted and un-extracted seeds as a component of animal feed, samples for nutrients and anti-nutrient were analyzed by Makkar and Becker (1997) and can be compared to soyabean Ologhobo *et al*. (2006). It has been reported that the crude protein content of extracted and un-extracted *Moringa* seeds values of 43.5 and 35.1%, respectively, suggesting that both the extracted and un-extracted seeds are good sources of protein for livestock. The crude protein and fibre contents of the extracted seeds were reported to be higher than those of the un-extracted seeds due to the loss of some cell soluble carbohydrates and lipids during the treatment with 80% ethanol (Makkar and Becker, 1997).

The crude protein, crude lipid and ash values of 36.4%, 65% and 8% respectively reported for the un-extracted seeds by Gupta *et al.* (2008) are in agreement with the values obtained by Makkar and Becker, (1997). It has been reported that the amino acid content (g/16gN) of un-extracted seeds is lower than that of extracted seeds due to the presence of a higher amount of non - protein nitrogen in the un-extracted leaves (4.7 vs. 2.7%). Chemical constituents of the crude aqueous extract of the seeds were found to be tannins, saponins, carbohydrates, flavonoids, cardiac glycosides, alkaloids, oxalates, steroids and terpenes. The crude

aqueous extract of the dried leaves contain the same chemical components like that of the fresh leaves except for the absence of steroids and terpenes (Kwaghe and Ambali, 2009).

2.3.4 Anti-Nutritional Factors in *Moringa oleifera* Seed

The use of *Moringa* in monogastric animal feeding could be limited by the presence of some anti-nutritional factors (tannin), which adversely affect protein and energy utilisation in broilers (Onu and Aniebo 2011; Ogbe and Affiku 2012). The anti-nutritional factors found in moringa include oxalates, tannins, saponins and flavonoids (Agwunobi *et al*., 2002). A great limitation to the use of un-extracted *moringa* seeds is the presence of anti-nutritional factors such as tannin, saccharide raffinose, stachyose nitrate (0.5mmol/100g), oxalate (4.1%), saponin (1.2%) and phytate (3.1%) which produce flatulence in *monogastric* (Reddy *et al.* 1982).

These anti-nutritional factors in un-extracted *moringa* seeds can be removed by extraction through soaking. These flatulence factors are determined after extraction in 80% aqueous ethanol (William, 1984). The seeds of *moringa* are reported to be rich in mineral and the presence of oxalates and phytates at concentration of 4.1% and 3.1% respectively, is likely to decrease the mineral bioavailability (Reddy *et al*., 1982). The level of extract in young leaves is always more than that in matured leaves. The leaves are an excellent source of protein and a very low source of fat and carbohydrates. They are also exceptionally good source of sulphur containing amino acids methionine and cystine (Kristin, 2000). Nutrient value of *Moringa* seeds can be increased through the addition of an enzyme (phytase) to break down the phytic acid, leading to increased absorption of the phosphorus in *Moringa*, Ogbe and Affiku (2012).

2.3. 4.1 Tannins

Tannins are naturally occurring plant polyphenols. Their main characteristic is that they bind and precipitate proteins. They can have a large influence on the nutritive value of many foods eaten by human and feedstuff eaten by animals (Hagerman and Klucher, 1996). Tannin is widely believed to be detrimental to livestock. However, recent researches have shown that it has some beneficial importance.

2.3.4.2 Toxic and anti-nutritional effect of tannins

Tannins induce negative response when eaten. These effects can be instantaneous detected as astringency or bitter taste or can have a delayed response related to anti-nutritional effects. Tannins reduce palatability because of its astringent or bitter taste which result in reduced feed intake thereby negatively affecting voluntary feed intake. Low palatability depresses feed intake and thus animal productivity. However, it should be noted that in many trials such as this, commercial tannin sources were used, and these types of tannins are usually more effective at lowering feed intake than naturally-occurring tannins (Hagerman and Klucher, 1996).

2.3.4.3 Toxicity of tannins to animals

Hydrolysable tannins are toxic to animals. The major lesions associated with hydrolysable tannins poisoning are hemorrhagic necrosis of the liver, and kidney damage with proximal tubelar necrosis. High mortality and morbidity were observed in animals fed with more hydrolysable tannins. Animals fed diets with a high level of tannin will experience depressed growth, damage of the mucosal lining of the digestive tract and low protein utilisation which increased excretion of proteins or amino acids may alleviate the anti-nutritional effects of tannins (Ahn, 1990).

Moderate levels of tannins in *Moringa* have been reported to have beneficial responses in animals, resulting in higher growth rates and milk yield (Hagerman and Klucher, 1996).

2.3.4.4 Saponins

Saponins have been reported to form complexes which decreases the availability of mineral for absorption or metabolism by West *et al.* (1998). Saponins from some plants have adverse effect on the growth of animals but those present in *moringa* seeds appear not to show haemolytic activity and humans consume them without apparent harm (Makkar and Becker, 1997). Saponins have bitter to taste and so can reduce palatability in livestock feed. Saponins however have a wide spectrum of activity as antifungal and antibacterial agents, lowering of blood cholesterol as well as inhibition of cancer cell growth (Michael, 2005).

2.3.4.5 Oxalates

Oxalates have been found to be useful for defense mechanism and a storage reserve for calcium (Smith, 1992). It has been reported that the presence of oxalic acid caused acute hypocalcaemia leading to rapid death, break down of red blood cells in animal. It may crystallize out in brain tissue and cause paralysis and other disorders of the central nervous system (Clerke and Clerke, 1995). Large amounts of oxalic acid consumption are liable to cause calcium and iron deficiencies Smith (1992).

2.4 Phytochemicals of *Moringa* and their Uses

An examination of the phytochemicals of *Moringa* species affords the opportunity to examine a range of fairly unique compounds (Fahey *et al.*, 2005). In particular, this plant family is rich in compounds containing the simple sugar, rhamnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates (Bennett *et al.*, 2003; Fahey *et al.*, 2005). Some of the compounds that have been isolated from *Moringa* preparations which are reported to have hypotensive, anticancer and antibacterial activity include 4-(4'-0-acetyl-a-L-rhamnopyranosyloxy) benzyl isothiocyanate, 4-(a-L-rhamnopyranosyloxy) benzyl isothiocyanate, niaomicin, pterygospermin, benzyl isothiocyanate, and 4-(a-L-rhamnopyranosyloxy) benzyl glucosinolate (Daxenbichler *et al.*, 1991; Fahey *et al.*, 2001; Bennett *et al.*, 2003; Mekonnen and Drager, 2003). Antioxidant activity of these compounds has also been reported (Win and Jongen, 1996, Ogbe and Affiku 2012.)

Seeds of *Moringa* have been reported to contain flavonoid pigments such as quercetin, kaempferol, rhamnetin, isoquercitrin and kaempferitrin (Nair and Subramanian, 1992). According to Foidl *et al.* (2001) extracts of *Moringa* seeds in 80% ethanol contain cytokinine-type hormones.

The flavonoids such as quercetin and kaempferol were identified as the most potent antioxidants in *Moringa* seeds. Their antioxidant activity was higher than the conventional antioxidants such as ascorbic acid, which is also present in large amounts in *Moringa* leaves (Siddhuraju and Becker, 2003).

The extracts of *moringa* seeds as reported by Siddhuraju and Becker (2003) also appear to have cancer preventive effect, when assayed by the differentiating activity against human promyelocytic leukaemia cells (HL-60). Seeds of *moringa* contain a glucosinolate that on

hydrolysis yields 4-(a-L-rhamnosyloxy)-benzyl isothiocyanate, an active bactericide and fungicide (Grubben and Denton, 2004). Duke (1983) reported that *Moringa* root-bark yield alkaloids: *Moringinine.*

Table 2: Phytochemical Constituents Isolated from *Moringa oleifera* Plant

Parts	Phytochemical constituents
Roots	4-(a-L-rhamnopyranosyloxy)-ebenzylglucosinolate and benzylglucosinolate
Stem	4-hydroxymellein, vanillin, p-sitosterone, octacosanic acid and P-sitosterol
Bark	4-(a-L-Rhamnopyranosyloxy)-Benzylglucosinolate
Whole gum exudates	L-arabinose, D-galactose, D-glucuronic acid, L-rhamnose, D-mannose, D-xylose and Leucoanthocyanin
Leaves	Glycoside niazirin, niazirinin and three mustard oil glycosides, 4-[4'-O-acetyl-a-L-rhamnosyloxy) benzyl] isothiocyanate, niaziminin A and B
Mature flowers	D-mannose, D-glucose, protein, ascorbic acid and polysaccharide
Whole pods	Nitriles, isothiocyanate, thiocarbanates, O-[2'- hydroxy-3' -(2 '-heptenyloxy)] - propylundecanoate, O-ethyl-4-[(a-1-rhamnosyloxy)-benzyl] carbarnate, methyl-p-hydroxybenzoate and P-sitosterol
Mature seeds	Crude protein, Crude fat, carbohydrate, methionine, cysteine, 4-(a-L-rhamnopyranosyloxy)-benzylglucosinolate, benzylglucosinolate, moringyne, momo-palmitic and di-oleic-triglyceride
Seed oil	Vitamin A, beta carotene, and precursor of vitamin A

Source: Mehnaz (2008)

2.5 Biochemical Evaluation of Oilseed Cakes

It has been established that protein concentrates used as supplementary source of protein in the diets of farm livestock differs widely in nutritive quality (Ologhobo, 1980, Fetuga, 1984). Hence there is a need for constant evaluation of its chemical and biological characteristics that are of nutritional importance.

2.5.1 Chemical Evaluation

The chemical methods used in evaluating the quality of oilseed cakes, are based on estimates of the heat damage of one form or another, to the protein. The chemical tests have been developed to serve as rapid means of predicting the quality, the prospects of carrying out a single test might not seem to be so useful.

2.5.2 Measurement of nitrogen solubility

The solvent action of dilute solution of salt proteins have been long recognised. The determination of protein by heat is of particular concern in oilseed cakes, since nearly all commercial processing involves the use of heat. One of the properties of a protein which is changed by heat denaturation is its solubility in water, or aqueous solution of salts, acids and alkalis (King *et al.* 1986). Ahn, (1990), Wiseman (1998), Madubuike (1992), Chikwedu and Obizoba (2003) have obtained good correlation between nitrogen solubility in 0.02N sodium hydroxide and protein quality of cottonseed cake, fish meal and soyabean meal, as measured by chick growth. In another study, Ketiku and Smith (1984) reported a correlation between rat growth and the percent of total nitrogen which was soluble in 3% sodium chloride solutions. Ruada *et al.* (2003); reported identical correlations between the growth response in rats and the solvent of 0.02N sodium hydroxide, 6N hydrochloric acid and 0.5N sodium chloride solution for protein supplements, which points to the fact that measurement of nitrogen solubility in a given solvent can be used as an indicator of the nutritive value of protein concentrates.

2.5.3 Measurement of amino acid availability

For the purpose of non-ruminant animal feeding, the most useful method of estimating the value of oilseed cake protein is the determination of the availability of certain amino acids, mainly lysine and methionine. Chemical estimation of available lysine and available methionine have

been adopted widely for use in laboratories. Available lysine defined as lysine with the epsilon - amino groups free, which was not reduced during processing, especially in cottonseed processing to cottonseed cake (Ologhobo, 1980). Values of available lysine have been correlated with the growth of chicks and a plot of the logarithm of the mean individual chick gains against available lysine content of diets was found to be a straight line (Nwanjo 2007). Smith (1982) reported significant correlations of available lysine with weight gain and protein efficiency ratio in rats. The chemical method which have been used in estimating available lysine content of proteins include that developed by Ologhobo and Fetuga (1988) which was based on the reaction of 1-fluoro-2, 4-dinitrobenzene (FDNB) with the free epsilon- amino groups of lysine, and gives a measure of the proportion of the total lysine which was available for use in the metabolic process of the animal. Available methionine can be determined by method described by Pieniazek *et al*. (1974). Essentially, this method involved preliminary hydrolysis of the protein concentrates with the enzyme pancreas to peptidase E. The hydropolysates were then reacted with sodium nitroprusside as described by McCarthy and Sullivan (1941), the resulting coloured complex was measured unetrically using DL-methionine as standard.

The availability of lysine and methionine have been measured, using the organic Tetrahymeno *pvriformis* simplified method which has been described by Scot *et al*. (1963). They also reported that the organism Streptococcus *zymogens* possesses an absolute requirement for methionine, leucine, isoleucine, arginine histioine, and valine, and has described a method for measuring the availability of these amino acids.

Bunyan and price (1992) reported the microbiological assay values for available methionine and tryptophan and chemically determined availability and biological values. However, Carpenter *et al*. (1973) have criticised the biological method on the basis that they often lead to over-estimation of available amino acids one to their high coefficient of variability and what the authors referred to as "systematic errors". It would appear therefore as though for available ammo acids, the chemical assays produce more reliable results than the microbiological methods.

2.5.4 Dye-binding methods

Boyne *et al*. (1971) described methods for determining the total acidic and basic groups of proteins based on their ability to combine with dyes in buffered acidic or alcoholic solutions.

They found the acid dyes particularly suitable for their purpose and orange G. binding proved to be especially satisfactory, they obtained correlations between orange G. binding and gross protein value (GPV) for whale and fish meals, but reported no correlations in a range of eleven groundnut cake. Daghir *et al.* (1979) have reported the amount of dye bound, to correlate directly with the amount of protein in the material. They also reported that the amount of dye bound depends on the number of reactive groups in the protein. This dye-binding capacity can be used to indicates the nutritional damage of proteins by heat treatment. Bunyan and Price (1992) reported a reduction in the dye-binding capacity of feedstuff protein due to over-heating and have reported the use of Remazol Brilliant blue binding, as an indicator of available lysine.

Frolich (1984) had advocated the use of Cresol Red, a plithalein dye, as a means of monitoring the heat treatment of soyabean meals and oilseed cakes. However, more recent work has shown that procedures involving binding reactive (available) lysine and Acid Orange 12 or Remazol blue, were more useful in dietary heat damage in proteins.

In general the dye-binding procedures have been found to be of some value in indicating the degree of heat damage in proteins, especially of plant origin to a greater extent than animal protein due to carbohydrate interference.

2.5.5 Protein dispersibility index

Protein dispersibility index (PDI) determines the dispersible protein in protein concentrates under the condition of the test (AOAC 1990). It is applicable to ground soyabean, whole or ground full-fat or extracted oilseed flakes, this involves the use of *a* formula; PDI% = % water dispersible protein X 100 % total protein

2.5.6 Measurement of chemical index

Due to the complication of gossypol in cottonseed cake protein, a chemical index (C.I.) to measure the nutritive value of the cake was proposed by Lyman *et al*., (1963); which involves the nitrogen solidibility of the cake in 0.02N Sodium hydroxide (NaOH) and its total gossypol content. They advanced a formula for the calculation of the chemical index as follow;

C.I. = % nitrogen soluble in 0.02N NaOH total gossypol (%) in the cake

In using the formula, a minimum total gossypol content of 0.85% is to be utilised, so that if the actual total gossypol content in the cake is less than 0.85%, then the value of 0.85% is to be used. The correlation of gossypol within material took account of solubility, but low nutritive value.

The authors also explained that since the free gossypol content of most cottonseed cake are low, the total gossypol content proved to be the more important factor in influencing the nutritional value of cottonseed cake.

2.5.7 Urease activity

Urease activity (U.A.), determine the activity of the residual urease in the soyabean products, under the condition of the test. It is applicable to soyabean meals, soy flour and to soyabean mill feeds, except when urea has been added. This can be determined by a titrimetric method (Ryan and Huisman.1986).

2.6 Biological Evaluation

The biological methods of evaluating the protein quality of oilseed cakes involved the use of laboratory animals such as rats, and small farm animals like the chicks. The test protein is fed to the selected animal under specified conditions and the response of the animal was measured by any of the methods as described below.

The problem of measuring the nutritive values of proteins involved not only the levels of amino acids in the protein, but also the availability of these amino acids to the animals. Therefore, animal experiments continued to be necessary in the study of protein quality. It is possible to use the protein under study as the sole source of protein and measure its ability to meet the animals total requirement for nitrogen and essential amino acid. Results of such a study might or might not indicate the ability of the protein to supplement a cereal based diet ahead of containing some protein of relatively low quality. In the bioassay procedures, the most common methods, are, those which employ growth rate to determine the adequacy of a given protein and those based on the ability of the assayed animal to utilise nitrogen (Ajenifuja, 1987).

2.6.1. Measurement of protein efficiency ratio;

Osborne and Mendel (1919) related the increase in weight to the amount of protein consumed by an animal, and this, postulated the concept of protein efficiency ratio (PER), which they defined as follows;

PER = gain in body weight (g) x protein consumed (g).

Protein efficiency ratio was shown to vary with dietary protein level, therefore, a suggestion was made that each proteins should be assayed at its optimum level. However, a dietary protein level of 10% is conventionally used in PER assays. Derse (1988) and Chapman *et al*. (1989), have shown that PER varied significantly with the age of the rats, while sex, rat strain and the length of the test period have also been shown to affect the values of PER (Jaisen, 1992). It is important to define clearly the conditions under which PER assays are carried out. The age of rats recommended for use in PER studies by AOAC (1990) is 21 to 28 days. Bender and Doell (1995) determined PER using a short experimental period of seven days and obtained wide variations in their results. The results have been criticised on the basis of the fact that they over looked the initial period (of about seven days) during which the animals were getting accustomed to the test diets and actually measured the adjustments of the rats to the diets. Chapman *et al*., (1989) showed that test periods of 3 days to weeks gave less variations in PER than when 1 to 2 weeks periods are employed, and most workers have adopted the 2-week period. The PER method has been criticised in three major areas. The first in the assumption that the gain in body weight has a contrast composition. It has been shown by Bresani (1994), the composition of body weight gain is influenced by the dietary components, the influence is mainly on the quality of body fat deposited, while the proportion of carcass nitrogen is not greatly influenced by variation in dietary fat or protein content.

Secondly, the 10% level of dietary protein used in PER assays has been criticized as being in excess of the need for this purpose. The third criticism of PER is that it makes no allowance for maintenance requirements while relating growth to total protein intake. Despite these criticisms, PER is continuously being used to evaluate the quality of proteins mainly because it is easy to operate.

2.6.2 Measurement of Net Protein Ratio

One of the major criticisms, of PER is that it does not include maintenance requirements in arriving at protein value. Daghir (1979) have attempted to overcome third default by introducing a method of evaluating protein quality based on Net Protein Ratio (NPR).The procedure involves feeding a group of weanling rats a diet containing 10% protein level using the test protein, and a comparative group of rats fed non-protein diet for a period of ten days. The difference between the weights of the two groups is used to calculate the NPR.

NPR = gain in weight - weight loss of test group (g) non-protein group (g) protein consumed (g)

2.6.3 Measurement of Gross Protein Value

This method was developed by Robertson *et al.* (1938) and was based on comparative growth response achieved in chicks as a result of supplementation of the test protein and that due to supplementation of casein, both measurements having been made over the growth achieved by a control group of chicks. The control group is fed a basal diet containing 8% crude protein for a period of two weeks, while the casein and the test groups receive the same basal diet supplemented with 3% casein and the test protein. The gross protein value (GPV) of the test protein is their calculated as the extra growth obtained with the supplement over the control divided by the amount of supplementation protein consumed.

This is then expressed as percentage of the corresponding figures obtained with casein as a supplement. Anwar (1980) modified this method by making allowance for the weight gained in gram per gram of protein intake from the control group as claimed that the results obtained by the modified method were better. Daghir *et a*l. (1979) in turn criticized Anwar's procedure as having based the calculation on 3% protein for the casein and the test diets, whereas the chicks had actually received 11% protein diet, and then compared to an 8% protein basal diet. They then proposed a modification of Anwar's procedure in which an 11% protein level was used for casein and the test diets, rather than the 3%.

GPV = gram increased weight per gram test protein x gram increased weight per gram casein

It is claimed that GPV is an assay which reflects the available lysine content of protein materials; (Boyne *et al.*, 1971). It is now known that such a claim in itself limits the practical applicability

of GPV, since higher protein levels under practical conditions will reduce the effect of differing lysine availability. Other limitations of the GPV method includes, the fact it gives values that can be compared with other proteins only in supplementation terms and the use of casein as standard is unsatisfactory for chicks, since casein is efficient in arginine, cystine and glycine, all of which are essential for the chicks.

2.6.4 Rat repletion method

The repletion method was developed by Cannon *et al.,* (1947). The procedure involved depleting rats of body protein for a given period by feeding a protein - free diet followed by repleting their body proteins on diets containing test proteins. The increase in weight of the depleted rats during the period of repletion was regarded as a measure of nutritive value of the proteins. In the development of the method, they had used adult rats, which meant that the repletion for their body weights by the test proteins could be regarded as meeting body weight maintenance only. Subsequent workers have modified the method by utilising growing rats and shortening the depletion period, to avoid starving to death (Ologhobo and Fetuga 1988).

2.6.5 Measurement of nitrogen balance

A more accurate evaluating protein quality could be obtained using results obtained from nitrogen balance trials. This is calculated by the difference between nitrogen intake and nitrogen excreted both in urine and faeces. Developed by Mitchell (1924); the nutritive value of the test protein to the animal is determined by the ability of the protein to cause positive nitrogen balanced in the animal. If the animal excretes in faeces and urine, as much as nitrogen, as it takes in the animal is said to be in nitrogen equilibrium but when the latter exceeds the former, the animal is said to be in negative nitrogen balance.

Nitrogen balance = I - (U + F)

Where I = nitrogen intake; u = urinary nitrogen; F = Faecal nitrogen.

Certain factors have been found to influence nitrogen balance studies and these includes age of rats, sex weight and colony differences (Ologhobo 1980). These workers also found that feed intake, faeces and urine collection and cross-contamination between faeces, urine and diet are additional variable which complicate nitrogen balance studies. Despite these limitations, the

nitrogen balance method appears to be desirable because it provides additional information on the utilisation and partronasing of dietary protein.

2.6.6 Digestibility method

Both nitrogen intake and that excreted are determined the retained nitrogen when related to nitrogen intake gives apparent digestibility.

Apparent digestibility (%) = nitrogen retained x 100/nitrogen intake.

But animals fed non-protein diets unexcreted nitrogen in the faeces and this is known as metabolic faecal nitrogen (MFN). In experiments involving the calculation of true digestibility, this is taken into consideration; True digestibility % =

100 nitrogen intake - (faecal nitrogen - nitrogen intake)

Attempts have been made to initiate the action of the mammalian system using *in vitro* enzymic digestion. The techniques often involved preliminary for grinding before the *in-vitro* test is performed and may not be exactly applicable to the original diet. Not only can be fulness of grinding affect nitrogen component remaining unchanged.

2.6.7 Biological Value

This is the percentage of the nitrogen absorbed which is retained by the animal. Growing rats are employed in the assay and thereby, measuring the requirement for both growth and maintenance. Reviewed by Mitchell (1924), a balance trial was conducted in which nitrogen intake, urinary and faecal nitrogen excretions were measured. The value obtained gave apparent biological value (ABV); thus;

Apparent BV = Nintake - (faecal N + Urinary N) x 100 Nintake - faecal N

In trials with animals on non-protein diets nitrogen is excreted both in faeces and urine known as metabolic faecal nitrogen (MFN) and endogenous urinary nitrogen (EUN), respectively. Since, these forms of nitrogen may not have arisen due to protein under test, corrections for such, have to be made in order to obtain the true biological value of the test protein. Since they represent

nitrogen which has been utilised as a result of maintenance their correction may give a more precise true biological value (TBV), of the test protein, thus;

TBV • Nintake - (faecal N-MFNH - (Urinary N - EUN) x 100 N intake - (faecal N - MFN)

In trials employed to determine BV, enough of the dietary protein should be provided by the test protein to allow for adequate nitrogen retention; but not in excess so as to minimize the amino acid catabolism which would depress the estimation of biological value. However, sufficient non-protein nitrogen should be provided to minimize the catabolism of protein to give energy. The diet must also be adequate in energy to prevent the utilisation of protein for energy.

2.6.8 Measurement of Net Protein Utilisation

Procedure for estimating the Net Protein Utilisation (NPU) was developed by Miller and Bender (1955). The procedure involves two groups of rats, one which is fed a non-protein diet and the other is fed the test protein diet at 10% protein level for ten days during which the feed intake of the animals are measured. The animals are killed after ten days and their body nitrogen determined on a sample of dried, ground carcass and the NPU is calculated from the formula;

$$NPU = \frac{\text{body N of test group - body N of non-protein group}}{\text{N consumed}} \times \frac{100}{\text{N consumed by test group}}$$

Summers and Fisher (1961) advocated the use of 13% protein level in diet for the determined of NPU. They explained that this level permitted sub-optimal growth while remaining sufficiently highest to enable the distinction between amino acid requirements for maintenance and growth. They said that lower protein levels might lead to erroneous conclusions as a result of disproportionate utilisation of certain amino acids for maintenance needs. The use of 10% protein level gave higher NPU values than when dietary protein levels exceeded 10%. Derse (1988) and Ryan (1986) proposed a method similar to NPU called liver protein utilisation (LPU); in this method the nitrogen in the liver is determined instead of the whole body nitrogen; this simplifies the method, since, measurement of liver nitrogen only would be easier to accomplished.

2.6.9 Serum metabolites; an indice of nutritive value

Bressani *et al*. (1994) pointed out that changes of the diet had marked effects on the body and blood composition of broiler chicks. Bender and Doell (1995) also showed that with age there were changes in the calorie to protein ratio in weight of broilers. The total serum protein represents the sum of numerous different proteins, many of which vary independently of each other; they consist of the various fractions of albumins and globulins (Smith 1982). Observations on human fed a protein deficient diets and results from laboratory animal experiment have revealed that to be able to adapt to their new conditions, the homeostatic mechanism of the body is activated in order to meet these new demands (Obizoba 1986); they reported that the proteins homeostatic is secured by the changes of the catabolic and synthetic rates in the body and the exchange of albumin between extravascular and intravascuter phases. The studies of Ifon (2008), showed albumin synthesis is not related to the amount of calories, but to soon as protein intake increases, the rate of synthesis increases immediately, whereas the catabolic rate remains the same for a long period. The increase of the total intravascular albumin mass can be explained through these findings; here retainment of the intravascular albumin mass in low protein diet was at the expense of the extravascular compartment (Said and Hegsted, 2009). They reported that, the total serum protein and albumin are low in kwashiorkor and normal in marasmus. Inspite of widen range studies, the value of the serum levels of these substances at the initial stage of the disease as a tool for early diagnosis, is still a matter for further investigation .The serum protein include mainly the albumin and globulin fractions of the plasma, since most of the fibrinoge was removed in the clotting process, which was incidental to the preparation of the serum (Ologhobo 1980).They reported that the liver synthesis all the components of serum total protein, except the immunoglobulins which are produced by the spleen. In liver dysfunction the components of serum protein produced by the liver are reduced in blood due to decrease synthesis. The functions of the serum proteins includes defence mechanism, this is done by the immunoglobulins in response to infections; also, they are involved in the maintenance of plasma anocotic pressure, this is the osmotic pressure excreted by the blood vessels in the maintenance of equilibrium of biological fluids such as salts and water. Also, the serum proteins maintain osmotic balance between the circulating blood and the tissue spaces.

2.7 Carcass Evaluation

In the production and processing of chickens, the determination should be of great interest, in satisfying a large part of meal demand in the Country. Consumers are interested in the amounts of edible meat obtained, processors are interested in the production of a bird that satisfies both and does it most efficiently.

Live-weight varies with sex, genetic quality temperature, quality of feed consumed and feed efficiency. The importance of live-weight is determined more by poultry weight and its stage of development than by its age. A young actively growing animal normally makes a large gain in live-weight per unit of feed consumed, than does an older one, that is, as a bird increase in weight, the gain it makes on a give quality of feed decreases (Hill and Renner, 1993). They also found that carcass fat increased when the energy content of the diet and energy intake increased. They observed improved growth and feed efficiency in chicks fed higher levels of energy at protein levels of 24 and 28%. They postulated that the calorie-to-protein of a diet is important for its influence on growth, feed conversion and carcass composition .The sex, strain, nutrition and management are important determinants of the live-weight of birds (Edwards, 1983). He obtained live-weights of 2305 and 1981g for males and females of Cobg chickens raised for twelve weeks. Whereas work with different strains; White Plymouth Rock, New Hanmpshire and cross bred birds gave live-weight of 1299 and 1532g at twelve weeks, respectively. In the topics reports by Oluyemi and Roberts (2000) gave 1000g and 1200g for female and male broilers at six weeks. It was observed that birds raised in the temperates are heavier than in tropics, due to genetics, management, and climatic factors.

The plucked weight could be said to be of much importance to the consumer than live-weight, since the feathers are so removed, and this will determine the amount of weight required by the consumer. The conformation of the chicken play a major role in plucked weight determination, (Znanieka *et al*., 1986). They also observed that feathers were about 6 to 8% of the body weight and tended to decrease with increasing body weight. Hayse and Marion (1993) reported values of 70.08 to 75.23% for eviscerated dressed carcass expressed as percentage of live-weight, while Job (1989), Ayorinde (1992) obtained an insignificant difference in the eviscerated weight of broilers raised on sweet-potato-maize diet. Essary *et al*. (1995) reported that varying dietary protein and fat levels did not appreciably influence dressing percentage of ten-week old

broilers. In an experiment conducted by Affifi and Rasheed (1996), they reported dressed weight as a percentage of liveweight using Rhode Island Red birds at two months, as 61.8% for males and 61.1% for females. Also that of Orr and Moran (1989) using 800 male and ten weeks of age, for males dressing percentage averaged 61.4, 66.6, 67.4, 68.2 and 69.7, respectively, while corresponding percentage for females were 64.7, 65.7, 65.9, 68.0 and 69.5, respectively. Kum (1993) gave dressing percentage values of 68.9 to 71.5%, after feeding broiler different levels of sweet potato-maize diets, this revealed the significant effect of diet on carcass dressing percentage, Consequently, he reported that unbalanced diet will result in a lower dressing percentage. While Ayorinde (1992) reported values of 66.7 to 87.42% as dressing percentage of birds fed varying energy-source diets. Lind (1997) found that dressing percentage increased significantly as the age at slaughter increased.

Relative weight of cut-parts (body parts) have been of major interest in recent years, this has prompted researchers to measure the weight of the different cut-parts of broilers and also their proportion to the dressed or eviscerated weight, the body parts that are mostly considered are the legs (thigh and drumstick), breast, wing, back and neck (Affifi and Rasheed 1996). Hayse and Marion (1993) found that the relative yield and percentage of the various parts have weight changed appreciably, they reported that although the market age of the broiler chicken has greatly changed, the proportion of the cut-up ready-to-cook chicken have remained constant.

Demand for poultry meat has developed in parallel with a tread of cutting the carcass into parts and selling the more valuable parts separately this has resulted in breast being marketed separately as a specially item in many different forms. They also reported that the body composition is influenced by altering the calorie-protein ratio, primarily but to its effect on appetite. The total edible meat has been shown as the most preferable measure of met quantity in the body (Brown *et al*., 1981). It is the total meat in the body, after its careful removal from all bones They also reported the percentage edible meat for cross bred broilers, Rhode Island Red, White Leghorn (light), White Plymouth Rock (heavy) and Colombian (male) roasters as 52.9, 53.8, 53.7, 62.2 and 60.1% respectively. In an experiment carried out by Znanieka *et al*. (1986) proportion of edible parts rose with increasing body weight and was at 1200g, 51.8% for males, 53.9% for females. At 1500g, 52.6 and 54.2% while at 1800g, 56.4 and 55.8%.

Gresser (1997) observed, that under present condition of feed quality, meat processing, the realistic maximum yield of edible meat from poultry is 74 to 75%. The weight of the eviscerated carcass and edible meat weight consist of the weight of many different parts which balance each other giving a linear relationship over all. It is a common assumption that plump appearance in broilers is associated with a higher percentage of edible meat, Hayse and Marion (1993). Ayorinde (1992) obtained a range of 56.09 to 60.32% as total edible meat related to liveweight of broilers. Scott and Darrow (1996) reported that the meat to bone ratio of certain pure breeds were New Hampshire (broiler type) 6.95, Plymouth white Rods (broiler type) 7.18 and White Plymouth Rocks (production type) 7.17.They also reported that, this ratio is affected by age and that it increases with increasing body weight.

In the studies of Marion and Woodroof (1995) and Affiku and Rasheed (1996) on the effect of diet on carcass composition, it was found that the level of dietary protein or fat did not affect total bone or meat yield, but that age has a significant effect on total bone. They reported that for a 16% protein level, the percentage bone and meal were 11.2% and 88.8%, while for 24%, corresponding values of 11.2% and 88.6% were reported for percentage bone and meat respectively. Hayse and Marion (1993) working with 8-week old broilers, obtained meat-to-bone ratio of 3.94 and 3.32 for females and makes respectively. The amount of abdominal fat pad shows the degree of excess deposited after the adipose tissue had been saturated with fat deposit underneath the skin. The degree of fat deposit in broiler is influenced by both nutritional and anti-nutritional factors; one of the most widely investigated in the calorie-to-protein ratio; diet with more energy gave more abdominal fat, while narrowing this ratio has generally been found to prevent excessive deposition of body fat (Rand *et al.*, 1987). They also reported that, the amount of fat in the carcass of chicks was inversely correlated with the calorie-to-protein ratio and with the ratio of protein intake to the growth rate. Brown (1981) has demonstrated that feeding of low energy-high protein diets acts to reduce the amount of carcass fat in the chicken. Summers *et al*. (1995) reported that the amount of carcass fat of chicks decreased with increasing levels of dietary protein and decreased level of dietary energy. Harms *et al*. (2003) demonstrated that the quality and type of dietary fats in the finisher diet influence the quantity of fat in the poultry processing efficient. Deaton *et al*. (2004) observed larger quantities of abdominal fat in broilers reared in cages when compared with broilers reared on the floor. Kubena *et al*. (2004) noted that older birds have more abdominal fat than those processed at an earlier age. They also

found that female broilers had a larger percentage of abdominal fat than the males and that higher rearing temperature increased the percentage abdominal fat in both males and females. Kubena *et al.* (2004) observed that the energy level of the starter diet to four weeks of age appeared to have a effect on abdominal fat present at seven and eight weeks of age .They reported that the amount of abdominal fat in male broilers exposed to early calorie restriction was reduced when the calorie-to-protein ratio of the diet was lowered. Deaton (2004) conducted work showing that carcass fat decreased and moisture content increased as the percentage protein and lysine in the diet increased. He reported that body protein was a linear function of body weight with carcass fat increasing as the protein level of the diet decreased. Carcass fat, at any body weight was greatest in birds fed low protein diets, the carcass fat increase was magnified by increasing the energy of the diet at any given protein level.

Generally, it has been observed that the more the edible meat present in a bird, the more desirable it is to consumer and the more economical and profitable to the poultry farmer

CHAPTER THREE

MATERIALS AND METHODS

CHEMICAL CHARACTERISATION OF RAW AND WATER – SOAKED *MORINGA OLEIFERA* SEEDS

3.1 Collection and Processing of Samples: Dried *Moringa oleifera* pods were harvested at Logun Area, Oluwo Village, Egbeda, Ibadan, Oyo State Nigeria. The seeds were cleaned and divided into four equal parts and each part soaked in 1kg/5litres of water for 1, 2 and 3 hours respectively.

The first seed container had no water as it was regarded as control (Treatment 1) while the other seed containers were allowed to soak for different hours as below:

Treatment 1: Control (without water)

Treatment 2: 1 hour soaked in water

Treatment 3: 2 hours soaked in water

Treatment 4: 3 hours soaked in water

After the different hours of soaking, the seeds were drained and sun-dried for three days, after which the seeds were milled using electronic blending machine to produce a fine powder. The powders were stored in four different plastic containers until ready for chemical analysis

3.2 Analysis of proximate compositions of *Moringa oleifera* Seed

3.2.1 Moisture Content Determination: Moisture content was determined by the loss weight that occurs when the sample was dried to a constant weight in the oven at 105^0C. The drying and weighing continue until a constant weight was achieved.

$$\textbf{\% Moisture} = \frac{\text{Weight of sample- weight of sample after drying} \times 100\%}{\text{Weight of Sample}}$$

3.2.2 Dry Matter: 2g of sample was weighed on a foil paper and wrapped differently according to their various hours and it was replicated twice. The samples were taken to the oven to dry for 24hours, after which the samples were kept in a dissicator to cool before weighing.

$$\textbf{\% Dry Matter} = \frac{\text{Final weight of sample (g)} - \text{initial weight of sample (g)}}{\text{Initial weight of sample (g)}} \text{ X } 100$$

3.2.3 Ash: Ash is the inorganic residue obtained by burning off the organic matter of seed stuff at 600^0c in Muffle furnace for 2 hours. 1g of *Moringa* seed powder was weighed into a pre-heated crucible, and replicated twice. The crucible was placed on heating mattle to char after which it was placed into muffle furnace at 600^0C for 2 hours or until whitish- grey ash was obtained. The crucible was then placed inside a dissicator to cool for about 30minutes before it was weighed.

$$\textbf{\% Ash} = \frac{\text{(Weight of crucible+ Ash)} - \text{(Weight of crucible)}}{\text{Weight of Sample}} \text{ X } 100$$

3.2.4 Crude Protein: 1g of *Moringa* seed powder was weighed into kjeldah flask, 17mls of concentrated Sulphuric acid and 1 kjeldah tablet was then added. Heat was applied in a fume cupboard slowly at first to prevent undue frothing, and digestion continued until the digesta became colourless. It was left to cool down completely and 100mls of distilled water was added. The digest flask was rinsed 2-3 times to the bulk. Distillation apparatus was streamed up and 5mls of the digest was added into the apparatus via a funnel and it was allowed to boil. 5mls of sodium hydroxide was added, 5mls of boric acid measured in a conical flask and distilled up to 50minutes until the colour was changed from blue to green on Hcl of 0.1 was added and read on

a capilary apparatus and the colour was changed back to blue. The titre value which was the value of the acid used was recorded and read with the formular below:

% Crude Protein = % N × 6.25.

3.2.5 Crude Fibre: 1g of dried MOS was weighed and transferred into a conical flask. 100ml of Trichloroacetic Acid was added and the solution was gently boiled for 40minutes. The boiled acid- sample mixture was then filtered through flask funnel fitted with filter paper. The remaining residue in the conical flask was then rinsed several times with warm water. The residue was then oven dried at 65^0C until a constant weight was achieved.

$$\% \text{ Crude Fibre} = \frac{\text{Weight of sample – weight of residue}}{\text{Weight of sample}} \times 100$$

3.2.6 Ether Extracts: One gramme of Moringa seed powder was weighed into a filter paper and the weight of the filter paper was known according to the number of hours and this was replicated twice. The sample was kept inside extractor flask and petroleum ether (60^0C) of 300mls was added in a soxhlet apparatus for about 6 hours after which the sample was oven dried to constant weight and the weight were recorded.

$$\text{Ether extract} = \frac{W_1 - W_2}{W_1} \times 100$$

W_1 = weight of sample before extraction

W_2 = weight of sample after extraction

3.3 Analysis of Mineral Composition of Raw and Water- Soaked *Moringa oleifera* Seed

Approximately 2g of *Moringa oleifera* seed powder was accurately weighed and wet-ashed using a mixture of 60% perchloric acid and nitric acid. The mineral constituents were determined, using separate lamps, by atomic absorption spectrophotometer, Perkin-Ellner 20g. Phosphorus was determined calorimetrically by the phosphovanado- molybdate method (AOAC, 1999).

3.4 Fibre Fractions Determination

One gramme of *Moringa oleifera* seed meal was accurately weighed into capsules secured with lids and 120ml H_2So4 with CTAB (Cetyl trimethylammonium bromide) weighing 49.04g

concentration and made up with 20% of distilled water and titrated. 20g of CTAB was dissolved into the sample and oven dried for five hours at 105^0C. The capsule was cooled to room temperature in a dissicator and later weighed. The sample was ashed in crucible. The capsule was placed in pre-dried and pre-weighed ashing crucible and ashing was done at 600^0C. The ashing crucible was cooled slowly at 20^0C and placed in a dissicator for 60 minutes.

Calculations for Acid Detergent Fibre:

$$\% \text{ ADF} = \frac{W_3 - (W_1 \times C) - (W_5 - W_4 - D)}{W_2} \times 100$$

W_1= Initial capsule weight (mg)

W_2 = Sample weight (mg)

W_3 = Capsule + residue weight (mg)

W_4 = Empty ashing crucible

W_5 = Total ash (mg)

C = Blank correction for capsule solubility

D = Capsule ash (mg).

Calculation for Neutral Detergent Fibre:

$$\% \text{ NDF} = \frac{W_3 - (W_1 \times C) - (W_5 - W_4 - D)}{W_2} \times 100$$

W_1 = Initial capsule weight (mg)

W_2 = Sample weight (mg)

W_3 = Capsule + residue weight (mg)

W_4 = Empty ashing crucible

W_5 = Total ash (mg)

C = Blank correction for capsule solubility

D = Capsule ash (mg)

Calculation for Acid Detergent Lignin

$$\% \text{ ADL} = \frac{W_3 - (W_1 \times C) - (W_5 - W_4 - D)}{W_2} \times 100$$

Where:

W_1 = Initial capsule weight (mg)

W_2 = Sample weight (mg)

W_3 = Capsule + residue weight (mg)

W_4 = Empty ashing crucible

W5 = Total ash (mg)

C = Blank correction for capsule solubility

D = Capsule ash (mg)

3.5 Qualitative Screening on Photochemical Constituents

3.5.1 Tannins: 1g of moringa seed sample was boiled in 20ml of distilled water in a test tube and filtered, 0.1% $FeCl_3$ was added to the filtered samples and observed for brownish green or blue black coloration an indication of the presence of Tannins.

3.5.2 Phlobatannins: 10 ml of aqueous extract of moringa seed sample was boiled with 1% HCl acid in a test tube or conical flask. If the sample of seed contained phlobatannins, a deposition of red precipitate will occur.

3.5.3 Saponins: 2g of moringa seed sample was boiled together with 20ml of distilled water in a water bath and filtered. 10ml of the filtered sample was mixed with 5ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing was therfore mixed with 3 drops of olive oil and for the formation of emulsion as an indication of the presence of saponins.

3.5.4 Terpenoids: 5 ml of aqueous extract of moringa seed sample was mixed with 2ml of $CHCl_3$ in a test tube 3ml of concentrated H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish brown coloration indicated that terpenoid constituent was present.

3.5.5 Glycosides: 1ml of concentrated H_2SO_4 was prepared in a test tube 5ml of aqueous extract from moringa seed sample was mixed with 2ml of glacial CH_3CO_2H containing 1 drop of $FeCl_3$. The mixture was carefully added so that the concentrated H_2SO_4 was underneath the mixture. If cardiac glycoside was present in the sample, a brown ring will appear indicating the presence of the glycoside constituent.

3.5.6 Alkaloids: 5g of moringa seed sample was prepared in a beaker and 200ml of 10% CH_3CO_2H in C_2H_5OH was added to the seed sample. Dark brown colour indicated the presence of alkaloids.

3.5.7 Oxalate: was determined by dissolving 2g of moringa seed sample in 100ml of distilled water separately, followed by the addition of 10ml of Hcl. Each was boiled for 1 hour, cooled

and then filtered. Each of the content was made up to 300ml with distilled water. Duplicate portion of each of the filtrate of 125 was taken into 5 different beakers and drops of methyl red indicator was added, followed by the addition of concentration NH_4OH solution drop wise until the test solution changes from pink to faint yellow colour.

3.5.8 Phenol: 2g of *Moringa oleifera* seed meal was weighed into extraction bottle, 2ml of distilled water was added followed by few drops of 10% ferric chloride was added, to 1ml of the sample. Formation of green colour indicated the presence of phenol.

3.6 Quantitative Analyses of Phytochemical Constituents of Raw and Water-soaked *Moringa oleifera* Seed Meal

3.6.1 Alkaloids: This was determined according to the method of Harborne (1973).

Method: 5g of *Moringa* Seed was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue remaining was the alkaloid, which was dried, weighed and the value obtained was recorded.

3.6.2 Saponins: This was determined according to the method of Obadoni and Ochuko (2001).

Method: 10g of *Moringa* Seed was weighed into a conical flask and 50ml of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 hours with continuous stirring at 55^0C. The mixture was filtered and the residue re-extracted with another 100ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at 90^oC. The concentrate was transfared into a 20ml separating funnel and 10ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while ether layer was discarded. The purification process was repeated. 30ml of n-butanol was added. The combined n-butanol extracts was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a

water bath. After evaporation, the sample was dried in the oven to a constant weight and the saponin content was calculated as percentage. By deducting the saponin content from the filter paper and multiplied by 100.

3.6.3 Phenols: This was determined using spectrophotometric

Method: 1g of *Moringa* Seed was weighed and the fat free sample was boiled with 50ml of ether for the extraction of the phenolic component for 15minute, 5ml of the extract was pipette into a 50ml flask, then 10ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrate amylalcohol were also added. The samples were made up to mark and left to react for 30minutes for colour development. This was measured at 505nm or wave length.

3.6.4 Phytic Acid: This was determined according to an indirect colorimetric method of Wheeler and Ferrel (1971). Determination of phytate by this method depends on an iron to phosphorus ratio of 4:6. Five gramme of *Moringa oleifera* seed of different treatments was extracted each with trichloro acetic acid. The phytate was precipitated as Ferric phytate and converted.

3.6.5 Cyanogens: This was determined by the method described by Knowles and Watkins (1950). Five gramme of *Moringa oleifera* seed was weighed into 20ml Conical flask. The sample was incubated for 16hours at 38^{o}c and later extracted with 95% methanol. Sample was then filtered using double layer of hardened filter paper and distillation was done with marham distillation apparatus. The extracted sample was transferred into a low-necked 500ml flask connected with a steam generator. This was steam distilled with saturated sodium bicarbonate solution contained in a 50ml conical flask for 1 hour. 1ml of starch indicator was added to 20ml each of the distillate and was titrated with 0.2N of iodine solution. The percentage hydro cyanide content was calculated.

3.6.6 Glucosinolates: This was evaluated using Buljet's reagent as described by El-Olemy *et al.,* (1994); One gramme of *Moringa* seed was soaked in 10ml of 70% alcohol for 2 hours and then filtered. The extract obtained was then purified using lead acetate and Na_2 HP Solution before the addition of freshly prepared Buljet's reagent (containing 95ml aqueous picric acid+ 5ml 10% aqueous NaOH). The difference between the intensity of colors of the experimental and blank (distilled water and Buljet's reagent) samples give the absorbance and was proportional to the concentration of the glucosinolates.

3.7 Spectrophotometric Determination of Fatty Acids in *Moringa oleifera* Seed Meal: 2g of *moringa* seed meal was weighed into 100ml Conical flask, 20ml of Benzene was added, shaking thoroughly to extract all the fatty acids. The mixture was transferred into a 250ml Separatory Funnels to separate the benzene extract from the aqueous extract. 5 ml aliquot of the benzene extract was pipetted into a 15ml test tube and 2ml of 10% copper acetate added to develop a blue colour. Standard solutions of each fatty acids were prepared in the range 0-10ppm from 100ppm stock solution of each fatty acid. Absorbance or Optical Density of sample extract as well as standard solutions of different concentrations were read on a spectrophotometer at a wavelength defined for each fatty acids. The % of each fatty acid was obtained using the formula below:

$$\%\text{Fatty Acids} = \frac{\text{Absorbance of sample x Gradient Factor of a specific fatty acid}}{\text{Wt. of sample X 10000}} \text{ X DF}$$

Where DF = Dilution Factor

3.8 Spectrophotometric Determination of Amino Acids in *Moringa oleifera* Seed Meal using Ninhydrin Chemical Reaction (By Moore and Stein (1954) modified by Schroeder *et al*. (1998).

One gramme of well ground moringa seed was weighed into a stoppered 250ml conical flask, 100ml of Hcl was added to the sample stoppered and heated to incubate for 16hours to hydrolysed the sample. The mixture obtained was filtered through a double layered Whatman No 42 filter paper into another 250ml conical flask and stoppered. The hydrolysate obtained was stored at -4^{o}C prior to analysis. 2ml of the hydrolysate was pipette into 30ml test tube .10ml of buffered ninhydrin reagent was added, heated in a boiling water bath for 15mintues ,cool to room temperature and 3ml of 50% ethanol was added immediately. 0-5ug/ml working standard amino acids were prepared from each standard solution of amino acids to get the gradients factor from the calibration curve for each amino acids. The working standards were heated with the buffered ninhydrin reagent as done with the samples hydrolysate above. The absorbance or transmittance of sample buffered heated hydroiysate and working standard were measured at the wavelength of colour developed by each essential amino acids.

Table 3: Fatty Acids Wave length

FATTY ACIDS	WAVE LENGTH
Lauric	640nm
Stearic	650nm
Palmitic	630nm
Oleic	660nm
Linoleic	660nm
Linolenic	680nm
Behenic	655nm
Myristic	655nm
Palmitoleic	685nm
Eicosenoic	645nm

Source**: Ologhobo (1980)**

Table 4: Amino Acids Wave Length

AMINO ACIDS	WAVE LENGTH	COLOUR
Histidine	460nm	Purple
Isoleucine	580nm	Light purple
Leucine	590nm	Purple
Lysine	450nm	Orange yellow
Methionine	525nm	Greenish yellow
Alanine	620nm	Blue
Valine	490nm	Greenish blue
Threonine	615nm	Bluish green
Phenyalanine	585mm	Greenish blue
Trytophan	565nm	Yellowish blue

Source**:** Schroeder *et al*. (1998)

3.9 Assessment of Dietary Protein Quality of Raw and Water-Soaked *Moringa oleifera* Seed Meal in Albino Rats.

3.9.1 Experimental Site

The experiment was carried out at the Rat House of the Department of Animal Science University of Ibadan.

3.9.2 Collection of Sample

Dried *Moringa oleifera* seed pods were harvested at Logun Village, Egbeda, Ibadan, while other feed ingredients used for this experiment were purchased from a reputable miller at, Orogun, Ibadan.

3.9.3 Experimental Design

Forty (40) weanling albino rats weighing 30-40g were transferred from the stock of Animal house of physiology, University of Ibadan to the Rat House of Department of Animal Science, University of Ibadan. The rats were weighed and randomly assigned on the basis of body weight into four dietary treatments of ten rats each in a completely randomized design experiment. Rats in each experimental animal group were housed singly in a well ventilated stainless metabolic cages and allowed access to one of the four experimental diets. The control T_1 received a standard diet as casein, T_2 received 10% of raw *moringa* seed meal,T_3 received 10% water - soaked Moringa seed meal and T_4 received a protein free diet. The trial consisted of a 5-day acclimatisation period followed by a 21-day collection period. Water and diet were offered *ad-libitum*. Each rat was weighed at the beginning and at the end of the 21 day feeding trial.

3. 9. 4 Experimental Layout

Diet 1: (Control) 10% casein

Diet 2: 10% Raw MOSM

Diet 3: 10% Water-Soaked MOSM

Diet 4: Protein free diet.

Table 5: Gross Composition of Experimental Diets fed to Albino Rats

Ingredient (%)	**T_1**	**T_2**	**T_3**	**T_4**
Corn starch	55.00	40.00	40.00	60.50
Ground nut oil	8.00	7.00	7.00	10.50
Sucrose	12.25	10.00	10.53	13.00
Casein	10.00	-	-	-
Raw *MOSM*	-	28.25	-	-
Water -soaked MOSM	-	-	27.72	-
Non nutritive cellulose	5.00	5.00	5.00	5.00
Glucose	6.00	6.00	6.00	6.00
Oyster shell	1.00	1.00	1.00	1.00
Di calcium phosphate	2.00	2.00	2.0	2.00
Table salt	0.25	2.50	2.50	2.50
Vit. Premix*	0.5	0.5	0.5	0.5
Total	**100**	**100**	**100**	**100**

** Composition of premix: Vit A 10,000001iu, Vit D_3 2000,00iu, Vit E 20,0000mg, Vit K_3 2,000mg, Vit B_1 3,000mg, Vit B_2 5,000mg, Niacin 4,5000mg, Calcium Pantothenate 10,000mg, Vit B_6, 4000mg, Vit B_{12} 20mg, Choline Chloride 300,000mg, Biotin 100mg, Manganese 50mg, Iron 300,000mg, Zinc 120,000mg, Copper 80,000mg, Iodine 15000mg, Cobalt 300mg, Selenium 120mg, Antioxidant 120,000mg.*

3.9.5 Management of Animals

One group of animals were sacrified on day 1 for carcass analysis. One group which represent the blanks represented the protein free diet. Predetermined levels of several proteins based on estimates of their probable nutritive value added to the diets of other groups as shown in table 5. The carcasses were kept frozen in plastic bags for later analysis.

The frozen carcasses were chopped or sliced with a cleaver into relatively small pieces, placed in 400ml beakers, and dried at 95^0 to constant weight to determine the Total Body Water (TBW), for 3 to 5 days. The dry carcasses were ground, mixed and 5g aliquot heated with 20ml of 50% sulphuric acid for 3 hours at approximately 100^0C. The hydrolysate was quantitatively transferred to a 500ml volumetric flask with water to made up to volume. Total Nitrogen (TN) of these solutions were determined by the automated Kjeldah procedure with the Auto Analyzer and Total Body Nitrogen (TBN) will be calculated.

From the body weights and the mean body composition of the animals sacrified on the first day, the initial body water and body nitrogen of each animal was calculated. These values were subtracted from those determined at the end of the experiment which yielded the change in body water and body nitrogen for each animal.

3.9.6 Feed Intake (FI)

The feed consumed was calculated by subtracting the weight of the feed offered to the rats from the weight of the left over feed.

3.9.7 Body Weight Changes (BWC)

Rats were weighted before the commencement of the experiment to know the initial weight and were subsequently weighted at the end of each week. Body weight changes were calculated by subtracting the weights in the preceding week from the weights of the succeeding week.

3.9.8 Protein Intake (PI) = Protein in the Diet (%) × Feed Intake

3.9.9 Protein Efficiency Ratio (PER)

This was expressed as the ratio of the average protein intake to the body weight gain.

$$PER = \frac{\text{weight gain(g)}}{\text{Protein intake (g)}}$$

3.9.10 Net Protein Ratio (NPR) = Weight gain +Weight Loss

Protein intake (g)

3.9.11 Net Protein Utilisation (NPU)

This was calculated by the difference in the body weight changes between the test group and the basal (protein free) group. This can be express through the equation below:

NPU = weight gain on test diet + weight loss on basal diet
Protein consumed by test group.

3.9.12 Feed Conversion Ratio (FCR)

This was expressed as ratio of the average feed intake to the weight gain over same period.

FCR = Feed intake
Weight gain

3.9.13 Biological Value (BV) = N intake – (feacal N-urinary N) X 100
N intake-feacal N

3.9.14 Haematological Parameters

The following were the haematological parameters that were measured; Packed Cell Volume (PCV)%, Haemoglobin concentration (g/dL)%, Red Blood Cells (RBC) ($x10^6$/1,),White Blood Cells (WBC) ($x10^3$/1), lymphocytes (%,), monocytes (%), eosinophils (%), neutophils (%), Mean Corpuscular Volume (MCV), Mean Corpusclar Haemoglobin (MCH) and Mean Corpusclar Haemoglobin Concentration (MCHC). Packed cell volume was determined using micro haematocrit method as described by Mitruka and Rawnsley (1971). Haemoglobin was determined using methamoglobin method as described by Mitruka and Rawnsley (1971).

Red Blood Cell and White Bloods Cells were determined using the improved Neubauer haemocytometer after the appropriate dilution (Schalm *et al.*, 1997).

Both lymphocytes, monocytes, eosinophils and neutophils were determined by scanning Gresham's stained slides in the classic manner (Schalm *et al.*, 1997).

The blood samples of the experimental animals were collected from the eye lid. New sterile needle was inserted into the vein and 1ml of blood was extracted and placed in a vacu-container test tubes containing Ethylene diramine tetra acetic acid (EDTA) to prevent coagulation. The packed cell volume (PCV) and haemoglobin (Hb) was determined using the micro haematocrit

method and cyanmethalmoglobin method respectively as described by Mitruka and Rawnsley (1971). Erythrocyte count (RBC) and Leucocyte count (WBC) were determined using the improved Neubauer haemocytometer after appropriate dilution (Schalm *et al.*, 1997).

3.9.15 Serum Biochemistry Analysis

The following are the serum biochemistry parameters that were determined: Albumin (Ab), Total protein (TP), Uric Acid (U), Glucose (G), Blood cholesterol, Globulin (GL), Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT).

Blood samples was obtained from three animals per replicate, (fifteen rats per treatment) by inserting a new sterile needle into the eye lid of the animals , 1ml of blood which was extracted and placed inside vacum container test tubes. The blood sample was separated from the serum and within two hours of collection was taken to the laboratory for serum biochemistry analysis. The vacum container tubes were centrifuged at 3000 rpm. for 10 minutes to separate the serum. A commercial diagnostic cholesterol reagent kit (Erba Diagnostic Mannasm Gmbh) was used for cholesterol determination, Total protein, Albumin, Globulin and urea, while ALT and AST were determined using spectrometric method as described by Rej and Hoder (1983), and McComb, *et al.* (1983) respectively.

Both the Alanine Amino Transferase (ALT) and Aspartate Amino Transferase was determined using spectrophotometric methods as described by Rej and Hoder (1983), and McComb, *et al.* (1983), respectively.

3.9.16 Histological Assay

Sections of the liver and kidney from five rats per treatment, one from each were collected and prepared into permanent slides according to the procedure outlined in Ewuola (2009).

The slides were used for histological evaluation and microscopic examination for lesions, vacuolations degenerations of hepatocytes, fatty changes in hepatocytes and necrosis (Pandey and Chauchan 2007).

Histology: A scale of 0 to 5 with 0 indicating no changes (0= no lesion, 1= mild lesion, 2= moderate lesion, 3= severe lesion) (Hoerr, 2003). Pathological changes were evaluated in the liver of birds which was scored based on descriptions of *Moringa oleifera* seeds induced hepatic pathology.

3.9.17 Statistical Analysis Data collected were subjected to analysis of variance (ANOVA) using SAS (2003) version and means were separated using Duncan Multiple Range Test Descriptive statistics using the software package of SAS (2003).

3.10 Replacement of Full-Fat Soyabean with Full-Fat *Moringa oleifera* Seed in the Diets Broiler Chickens

3.10.1 Experimental Site:

This experiment was carried out at the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria.

3.10.2 Sources of Test Ingredient

Dried *Moringa oleifera* seeds were harvested in Logun Area , Oluwo Village, Egbeda, Ibadan and other feed ingredients used for this experiment was purchased from Blessed Feeds (Nig.) Enterprises, Orogun, Ibadan.

3.10.3 Processing of the Test Ingredients

Collection and Processing of Samples: Dried *Moringa oleifera* pods were harvested at Logun Area, Oluwo Village, Egbeda, Ibadan, and Oyo State. The seeds were separated from the pods and then cleaned. Ten litres of water was poured into 2kg of moringa seed containers for three hours soaking. After three hours of soaking, the seeds were sieved to drained the water and spread on a clean new mat to sun – dried for three days, after which the seeds were milled using a clean, electronic blending machine. The grinding was repeated continually until a fine powder was obtained to ensure homogenecity.

3.11 Composition of the Experimental Treatments

Five treatments were used for this experiment. The treatment containing 100% full-fat soyabean was taken as the control (T_1), Treatment two (T_2) contained 25% three hours water-soaked moringa seed meal, treatment three (T_3) contained 50% three hours water-soaked moringa seed meal, treatment four (T_4) contained 75% three hours water-soaked moringa seed meal and

treatment five (T_5) contained 100% three hours water-soaked moringa seed meal. The dietary composition used for this experiment is shown on Table 6.

3.11.1 Experimental diets layout:

T_1 - (Control) 100% FFSB + 0% MOSM

T_2 - 75% FFSB + 25% Water-soaked MOSM

T_3 - 50% FFSB + 50% Water-soaked MOSM

T_4 - 25% FFSB + 75% Water-soaked MOSM

T_5 - 0% FFSB + 100% Water-soaked MOSM

Table 6: Composition of Broiler Starters Fed Water Soaked *Moringa oleifera* Seed

Ingredients (%)	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**
Maize	48.30	48.30	48.30	48.30	48.30
Fish meal	2.00	2.00	2.00	2.00	2.00
Full fat soyabean	35.00	26.25	17.50	8.75	0.00
Moringa seed meal	0.00	8.75	17.50	26.25	35.00
Wheat offals	10.00	10.00	10.00	10.00	10.00
Dicalcium Phosphate	2.00	2.00	2.00	2.00	2.00
Limestone	1.55	1.55	1.55	1.55	1.55
Table Salt	0.30	0.30	0.30	0.30	0.30
Vitamin Premix*	0.25	0.25	0.25	0.25	0.25
L-lysine	0.35	0.35	0.35	0.35	0.35
DL-Methionine	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100

**Composition of premix: Vit A 10,000001iu, Vit D_3 2000,00iu, Vit E 20,0000mg, Vit K_3 2,000mg, Vit B_1 3,000mg, Vit B_2 5,000mg, Niacin 4,5000mg, Calcium Pantothenate 10,000mg, Vit B_6, 4000mg, Vit B_{12} 20mg, Choline Chloride 300,000mg, Biotin 100mg, Manganese 50mg, Iron 300,000mg, Zinc 120,000mg, Copper 80,000mg, Iodine 15000mg, Cobalt 300mg, Selenium 120mg, Antioxidant 120,000mg*

Table 7: Calculated Nutrient Composition of Broiler Starter Fed *Moringa oleifera* Based Diets

Components (%)	T_1	T_2	T_3	T_4	T_5
Crude protein	22.96	21.80	21.70	22.83	22.11
Crude fibre	4.04	4.02	3.95	3.98	3.81
Ether extract	4.04	4.07	4.10	4.15	4.00
Calcium	1.04	1.10	0.99	1.10	1.00
Phosphorus	0.52	0.58	0.51	0.59	0.57
Lysine	0.94	0.97	1.01	1.00	1.04
Methionine	0.45	0.46	0.39	0.32	0.35
ME (Kcal/kg)	3020.27	3010.12	3010.19	3008.72	3004.60

Table 8: Composition of Broiler Finishers Fed Water-Soaked *Moringa oleifera* Seed

Ingredients (%)	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**
Maize	50.00	50.00	50.00	50.00	50.00
Wheat offals	14.60	14.60	14.60	14.60	14.60
Full fat soyabean	20.00	15.00	10.00	5.00	0.00
Moringa seed meal (soaked)	0.00	5.00	10.00	15.00	20.00
Fish meal	1.00	1.00	1.00	1.00	1.00
Palm kernel cake	7.00	7.00	7.00	7.00	7.00
Palm oil	2.00	2.00	2.00	2.00	2.00
Dicalcium Phosphate	2.50	2.50	2.50	2.50	2.50
Limestone	1.75	1.75	1.75	1.75	1.75
Table Salt	0.30	0.30	0.30	0.30	0.30
Vitamin Premix*	0.25	0.25	0.25	0.25	0.25
L-lysine	0.35	0.35	0.35	0.35	0.35
DL- Methionine	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**

×*Composition of premix: Vit A 10,000001iu, Vit D_3 2000,00iu, Vit E 20,0000mg, Vit K_3 2,000mg, Vit B_1 3,000mg, Vit B_2 5,000mg, Niacin 4,5000mg, Calcium Pantothenate 10,000mg, Vit B_6, 4000mg, Vit B_{12} 20mg, Choline Chloride 300,000mg, Biotin 100mg, Manganese 50mg, Iron 300,000mg, Zinc 120,000mg, Copper 80,000mg, Iodine 15000mg, Cobalt 300mg, Selenium 120mg, Antioxidant 120,000mg*

Table 9: Calculated Nutrient Composition of Broiler Finisher Fed *Moringa oleifera* Seed Based Diets

Components (%)	T_1	T_2	T_3	T_4	T_5
Crude protein	19.31	19.25	19.01	18.92	18.96
Crude fibre	4.22	4.56	4.92	5.18	5.25
Ether extract	3.46	3.55	3.64	4.03	4.13
Calcium	0.97	0.99	1.00	1.02	1.08
Phosphorus	0.65	0.67	0.67	0.70	0.74
Lysine	0.98	0.97	1.00	1.02	1.02
Methionine	0.44	0.45	0.48	0.47	0.51
ME (Kcal/kg)	3103.46	3096.57	3082.16	3001.43	2963.3

3.12 Experimental Procedures and Processes

Two hundred and fifty (250) day old broiler chicks were obtained from a reputable farm for this experiment. The experiment lasted eight weeks (56 days) which included an initial two (2) weeks of brooding period. On arrival, the chicks were individually weighed and randomly allotted to five dietary treatments of 50 chicks per treatment, and each treatment replicated 5 times (10 chicks per replicate) in a completely randomised design. Daily feed intake and weekly record of weight gain were monitored. The chicks were offered different feeds according to each treatment and clean water was given *ad libitum*. Routine vaccination procedures were followed.

3.13 Management of the Experimental Birds

The birds were reared in deep litter system and the floor was covered with wood shavings. The brooder house, feeders and drinkers were properly cleaned and disinfected prior to the study. The birds were raised in ambient temperature except during the first two weeks of brooding and adequate ventilation was provided. At the first two weeks, coal pots were used to supply heat.

3.14 Vaccination and Medication

On arrival, the chicks were served anti-stress dissolved in distill water for five days. At day 7, chicks were administered Lasota (against viral disease) in drinking water, while at day 14, gumboro vaccine was given to the chicks. At day 21, anti-stress and antibiotics was offered to the chicks. Used liters were changed weekly. Water spillage on the litter was minimised.

3.15 Data Collection

3.15.1 Chemical Analysis; The test samples, *Moringa oleifera* seed and the experimental diets were subjected to proximate analysis to determine the crude protein, crude fibre, ether extract,

ash and dry matter by the procedure of the association of the officials of analytical chemists (AOAC. 1990).

3.15.2 Feed Intake

Feed intake was collected by weighing a known quantity of feed given to each replicate and subtracting the reminant from it for each replicate. The feed intake was recorded on a daily basis and added up to give the total feed consumed per replicate for the 8 weeks.

3.15.3 Weight Gain

Birds were weighed individually at the beginning of the experiment and weekly weight gain was obtained by subtracting the weights of the previous week from the current week.

3.15.4 Feed conversion ratio (feed to gain ratio)

This was calculated by dividing the feed consumed by the weight gain for a given period of time and this was done on a weekly basis.

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Average feed intake (g)}}{\text{Average body weight gain (g)}}$$

3.16 Other Parameters Measured

3.16.1 Blood Parameters Measurement and Serum Biochemistry Analyses

Blood parameters and serum biochemistry were measured on the birds at the 56^{th} day. 75 birds were used of 15 birds per treatment and replicated three times.

3.16.2 Haematological Parameters

The following were the haematological parameters that were measured; Packed Cell Volume (PCV)%, Haemoglobin concentration (g/dL)%, Red Blood Cells (RBC) ($x10^6$/1,),White Blood Cells (WBC) ($x10^3$/1), lymphocytes (%,), monocytes (%), eosinophils (%), basophils (%), heterocytes (%), Packed cell volume was determined using micro haematocrit method as described by Mitruka and Rawnsley (1971). Haemoglobin was determined using methamoglobin method as described by Mitruka and Rawnsley (1971).

Red Blood Cell and White Bloods Cells were determined using the improved Neubauer haemocytometer after the appropriate dilution (Schalm *et al.*, 1997).

Both lymphocytes, monocytes, eosinophils, basophils and heterocytes were determined by scanning Gresham's stained slides in the classic manner (Schalm *et al.*, 1997).

The blood samples of the experimental birds were collected from the wing vein. New sterile needle was inserted into the vein and 1ml of blood was extracted and placed in a vacu-container test tubes containing Ethylene diramine tetra acetic acid (EDTA) to prevent coagulation. The packed cell volume (PCV) and haemoglobin (Hb) was determined using the micro haematocrit method and cyanmethalmoglobin method respectively as described by Mitruka and Rawnsley (1971). Erythrocyte count (RBC) and Leucocyte count (WBC) were determined using the improved Neubauer haemocytometer after appropriate dilution (Schalm *et al.*, 1997).

3.16.3 Serum Biochemistry Analysis

The following are the serum biochemistry parameters that were determined: Albumin (Ab), Total protein (TP), Uric Acid (U), Glucose (G), Blood cholesterol, Globulin (GL), Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT) and Alanine Phosphate (ALP).

Blood samples was obtained from three birds per replicate, (fifteen birds per treatment) by inserting a new sterile needle into the wing vein of the birds , 1ml of blood which was extracted and placed inside vacum container test tubes. The blood sample was separated from the serum and within two hours of collection was taken to the laboratory for serum biochemistry analysis. The vacum container tubes were centrifuged at 3000 rpm. for 10 minutes to separate the serum. A commercial diagnostic cholesterol reagent kit (Erba Diagnostic Mannasm Gmbh) was used for cholesterol determination, Total protein, Albumin, Globulin and urea, while ALT and AST were determined using spectrometric method as described by Rej and Hoder (1983), and McComb, *et al.* (1983) respectively.

Both the Alanine Amino Transferase (ALT) and Aspartate Amino Transferase was determined using spectrophotometric methods as described by Rej and Hoder (1983) and McComb *et al.* (1983), respectively.

3.16.4 Immune Responses

Blood samples were collected from five chicks treatment through the jugular vein at week four. Serum was separated by centrifugation (8000 rpm for 5minutes) and antibodies specific for IBD

virus were detected in the sera of chicks using an ELISA kit (PROFLOKR synbiotics Corporation, CA, USA) according to the manufacturer's instructions. One hundred microliters from each sample was used for the assay. ELISA absorbency was measured at 405nm using and ELISA reader (Sunrise absorbance reader, TECAN) by standard procedures (Snyder *et al*., 1984), to monitor the effect of the experimental diets on the immune response of the birds.

3.16.5 Histological Assay

Sections of the liver, kidney and heart from five birds per treatment, one from each were collected and prepared into permanent slides according to the procedure outlined in Ewuola (2009).

The slides were used for histological evaluation and microscopic examination for lesions, vacuolations degenerations of hepatocytes, fatty changes in hepatocytes and necrosis (Pandey and Chauchan 2007).

Histology: A scale of 0 to 5 with 0 indicating no changes (0= no lesion, 1= mild lesion, 2= moderate lesion, 3= severe lesion) (Hoerr, 2003). Pathological changes were evaluated in the liver of birds which was scored based on descriptions of *Moringa oleifera* seeds induced hepatic pathology.

3.16.6 Digestibility Trial

Digestibility were measured using the direct method of digestibility determination and this was be done by selecting Ten Birds, Two (2) from each treatment and were placed in metabolic cages and they were fed with experimental diet of known weight, water was provided *ad libitum*. The initial weights were taken and the feed given were weighed while on trial feeding for two days to adjust them to the caging.

From the 3rd day till 6th day, their weight were taken daily before feeding; feed remains and their droppings were also collected and weighed. The feaces collected were stored in the freezer so that the moisture content will not be lost and also for preservation till the end of trial. At the end of digestibility trial all the droppings collected were pooled and aliquot were taken for analysis. The digestibility coefficient for each dietary treatment were calculated.

3.16.7 Carcass Characteristics

At the end of the experiment, ten (10) birds were slaughtered by severing the jugular veins with sharp knife.

The live weight was taken before slaughtering. The slaughtered birds were de-feathered and weights were recorded. The dressed weight was taken after eviscention. The head, neck, shank, wings, breast, back, thigh, drumstick, liver, heart, abdominal fat, spleen and gizzard were also weighed.

3.16.8 Organoleptic Test

Organoleptic test was carried out on the experimental animals to determine their quality in terms of Taste, Color, Tenderness, Juiciness and the Overall Acceptability of the meat by a taste of panel.

This was done by cutting meat sample from the breast muscle of birds from each treatment, the meat samples were cut into Ten (10) small pieces which was tied in transparent nylon and labeled properly. The pieces of meat were cooked without salt in boiling water for 20 minutes.

After cooling each meat was cut into 10 pieces and served to ten taste judges one treatment after the other. After each treatment the judges were served cracker biscuits which neutralised their taste bud before taking another treatment also after each treatment the judges recorded their result on questionnaires that were given to them.

3.16.9 Statistical Analysis Data collected were subjected to analysis of variance (ANOVA) using SAS (2003) version and means were separated using Duncan Multiple Range Test Descriptive statistics using the software package of SAS (2003).

CHAPTER FOUR

RESULTS

4.1 Proximate Compositions of Raw and Water-Soaked *Moringa oleifera* Seed

The proximate compositions of the raw and water-soaked *Moringa oleifera* seed are shown in table 10. The dry matter values ranged between 95.30 and 98.06% which was below 10% moisture content, the crude protein contents ranged between 35.20 and 36.08% where three hours water-soaked *Moringa oleifera* seeds obtained the highest mean value. Crude fibre contents were 10.20, 11.60, 11.95 and 12.55% for raw, one hour, two hours and three hours water-soaked MOS, respectively. The ether extract ranged between 12.92 and 14.10% while total ash was higher (5.00%) in the raw MOS and two hours water-soaked MOS gave the least value (2.20%). The nitrogen free extract values ranged between 34.38 and 36.36% where the raw Moringa seed had the least value and the highest value was observed in two hours-water soaked MOS.

4.2 Mineral Compositions of Raw and Water-Soaked *Moringa oleifera* Seed

Table 11: showed the mineral compositions of the raw and water-soaked *Moringa oleifera* seed which was expressed in mg per 100g of the major minerals (calcium, phosphorus, potassium, magnesium and iron). Calcium values ranges between 0.42 and 0.44mg/100, which was most abundant in two-hour water-soaked MOS, while one hour water-soaked MOS had the least value. The highest phosphorus value was obtained in the raw MOS (0.40mg/100), while the values of the soaked MOS ranges between 0.34 and 0.37mg/100. Potassium ranged between 0.92 and 0.95mg/100, magnesium between 0.42 and 0.54mg/100 and iron between 179.35 and 188.30 (ppm). Three hour water-soaked MOS was particularly low in magnesium (0.42 mg/100) and iron (179.35ppm) especially when compared with the un-soaked moringa seed. With these

minerals values obtained, its indicated a greater abundance of these minerals in water-soaked *Moringa oleifera* seed, though they are however better supplied in the un-soaked MOS.

4.3 Phytochemicals Content of Raw and Water – soaked *Moringa oleifera* Seed

Table 12 showed the qualitative screening of raw and water-soaked *Moringa oleifera* seeds. From this table, ten phytochemicals were screened to detect the anti-nutritional factors present in the raw and water-soaked *Moringa oleifera* seeds. Out of the 10 phytochemicals (alkaloids, sapponins, tannins, phenols, flavonoids, phytates, cyanogens, terpenoids, glucossinolates, and oxylates) screened, it was observed that 6 (Alkaloids, sapponins, phenols, phytates, cyanogens and glucossionalate) of them were positive while 4 (Tannins, flavonoids, terpenoids and oxylates) were negative in both the raw and water-soaked MOS.

The levels of anti-nutritional factors present the in raw and different hours of water-soaked *Moringa oleifera* seeds are presented in Table 13. Treatment 1 (control) had the highest values of 0.37, 1.17, 0.06, 4.43, 1.37 and 66.25 μ/mg for alkaloids, saponins, phenols, phytates, cyanogens and glucosinolates respectively, which was significantly different from other treatments, while treatment 4 (3 hours water-soaked), had the least values except for glucosinolates where treatment 3 (2 hours water-soaked), had the least values of 51.83 (μ/mg).

4.4 Fiber Fractions Determination of Raw and Water – soaked *Moringa oleifera* Seed

Table 14 shows the results of fibre fractions of *Moringa oleifera* seed meal. From this results it was observed that there were significant ($P<0.05$) differences among the fibre fractions determinations. As the soaking time increased all the fibre fractions also increased. On the values of Neutral Detergent Fibre (NDF) which ranges from 34.82-43.18% for unsoaked MOS (T1) to 3 hours water-soaked (T4). The values for Acid Detergent Fibre (ADF) obtained in the un-soaked MOS (24.17%) was significantly ($P<0.05$) lower than the soaked MOS with the values of 27.57, 31.95 and 32.36% for 1 hour, 2 hours and 3 hours water-soaking respectively. The Acid Detergent Lignin (ADL) followed same trend with the values of 10.73, 15.00, 16.34 and 16.70% for unsoaked (T1), 1 hour (T2) 2 hours (T3) and 3 hours (T4) respectively. This showed that there were increases in values of the fibre fractions as the soaking time increased.

Table 10: Proximate Compositions of Raw and Water-Soaked *Moringa oleifera* Seed

PARAMETERS (%)	T1	T2	T3	T4	Mean	SD
Dry Matter	95.30	96.16	98.06	96.14	96.42	0.68
Crude Protein	35.40	35.25	35.73	36.08	35.62	0.72
Crude Fibre	10.20	11.60	11.95	12.55	11.58	2.55
Ether Extract	14.10	12.97	12.96	12.92	13.24	2.21
Ash	5.00	4.10	2.20	2.41	3.43	1.74
Nitrogen free extract	35.15	36.09	35.36	34.38	35.25	0.36

T1= Raw MOS, T2= 1 Hour water-soaked MOS, T3= 2 Hours water-soaked MOS, T4= 3 Hours water-soaked MOS

MOS: *Moringa oleifera* seeds

SD: Standard Deviation

Table 11: The Mineral Composition of Raw and Water – soaked *Moringa oleifera* Seed

PARAMETERS (mg/100)	T1	T2	T3	T4	Mean	SD
Calcium	0.43	0.42	0.44	0.43	0.43	0.04
Phosphorus	0.40	0.37	0.35	0.34	0.37	0.09
Potassium	0.95	0.92	0.93	0.93	0.93	0.06
Magnesium	0.54	0.46	0.44	0.42	0.47	0.12
Iron (ppm)	188.30	187.34	184.57	179.35	184.89	0.65

T1= Raw MOS, T2= 1 Hour water-soaked MOS, T3= 2 Hours water-soaked MOS, T4= 3 Hours water-soaked MOS

MOS: *Moringa oleifera* seeds

SD: Standard Deviation

Table 12: Results of Qualitative Screening of Phytochemicals in Raw and Water Soaked *Moringa oleifera* Seed

PARAMETERS (%)	T1	T2	T3	T4
Alkaloids	+	+	+	+
Saponin	+	+	+	+
Tannin	–	–	–	–
Phenols	+	+	+	+
Flavonoids	–	–	–	–
Phytates	+	+	+	+
Cyanogenes	+	+	+	+
Terpenoids	–	–	–	–
Glycosides	+	+	+	+
Oxalates	–	–	–	–

T1= Raw MOS, T2= 1 Hour water-soaked MOS, T3= 2 Hours water-soaked MOS, T4= 3 Hours water-soaked MOS

MOS: *Moringa oleifera* seeds

Table 13: Phytochemical Composition of Raw and Water -Soaked *Moringa oleifera* Seed

PARAMETERS (%)	T1	T2	T3	T4	Mean	SD
Alkaloids	0.37	0.20	0.11	0.07	0.19	0.05
Saponins	1.17	0.68	0.52	0.42	0.70	0.28
Tannins	0.06	0.06	0.05	0.05	0.06	0.02
Phytates	4.43	4.32	3.86	3.70	4.08	0.75
Cyanogens	1.37	1.18	0.93	0.93	1.10	0.54
Glucosinolates	0.66	0.59	0.51	0.50	0.57	0.08

T1= Raw MOS, T2= 1 Hour water-soaked MOS, T3= 2 Hours water-soaked MOS, T4= 3 Hours water-soaked MOS

MOS: *Moringa oleifera* seeds

SD: Standard Deviation

Table 14: Fibre Fractions of Raw and Water-Soaked of Raw and Water – soaked *Moringa oleifera* Seed

PARAMETERS (%)	T1	T2	T3	T4	Mean	SD
Neutral detergent fibre	34.82	39.83	43.11	43.18	40.24	0.58
Acid detergent fibre	24.17	27.57	31.95	32.36	29.76	0.25
Acid detergent lignin	10.73	15.00	16.34	16.70	14.69	1.17

T1= Raw MOS, T2= 1 Hour water-soaked MOS, T3= 2 Hours water-soaked MOS, T4= 3 Hours water-soaked MOS

MOS: *Moringa oleifera* seeds

SD: Standard Deviation

4.5 Amino Acids Determination of Raw and Water – soaked *Moringa oleifera* Seed

Table 15 shows the results of amino acid determinations of raw and water-soaked *Moringa oleifera* seeds. There were significant variations among the treatments. histidine ranged between 0.75 and 0.91%, isolecine between 0.48-0.57%, leucine 0.31 and 0.44%, lysine 0.38 and 0.57, methionine 0.62 and 0.75%, phenylalanine 0.15 and 0.19%, threonine 0.21 and 0.29, tryptophan 0.42 and 0.62%, valine 1.42 and 1.75% and alanine 0.23 and 0.35%.

The histidine, isolecine, leucine, phenylalanine and threonine values were relatively higher in 3 hours water-soaked (T4) than other treatments followed by 2 hours water-soaked MOS (T3), while the unsoaked MOS (T1) gave the least values. This result implies that *Moringa oleifera* seed is not deficient in all these amino acids when compared with soyabean but variations occurred among the treatments.

4.6 Fatty Acids Determination of raw and water – soaked *Moringa oleifera* seed

The percentage fatty acid compositions of raw and water-soaked *Moringa oleifera* seeds are presented in table 16. Ten fatty acids were analysed for which include: Oleic acid, behenic acid, palmitic acid, steric acid, linolenic acid, myristic acid, palmitoleic acid, eicosenic acid, lignoceric acid and arachidic acid, with values ranges from 40.15 and 54.43%, 6.54% and 7.00, 5.38 and 6.50, 4.25 and 5.31%, 0.18 and 0.22%, 0.46 and 0.53%, 2.00 and 2.30%, 1 75 and 1.95%, 0.92 and 1.01, 3.25 and 4.20%, respectively. Oleic acid was appeared most abundant, which was higher in the un-soaked MOS (T1) (54.43%), followed by behenic and palmitic acids and the un-soaked MOS (T1) containing the largest amount of 7.00% and 6.50% respectively. 3 hours water-soaked MOS (T4) obtained the most abundance steric acid, myristic acid, palmlitolic acid, and eicosenic acid with the values of 5.31%, 0.58%, 2.20% and 1.95% respectively while 1 hour water-soaked MOS (T2) obtained the highest relative lignoceric value (1.20%). Also the un-soaked (T1) had the most abundant linolenic (0.22%) and arachidic acid (4.20%) while 2 hours water-soaked MOS (T3) obtained the least values (0.16 and 2.55%) respectively.

Table 15: Amino Acid Profile of Raw and Water -Soaked *Moringa oleifera* Seed

Amino acids (%)	T 1	T2	T3	T4	Mean	SD
Histidine	0.75	0.87	0.83	0.91	0.82	0.08
Isolecine	0.50	0.48	0.52	0.57	0.54	0.14
Leucine	0.31	0.35	0.43	0.44	0.38	0.18
Lysine	0.38	0.46	0.49	0.57	0.48	0.26
Methionine	0.62	0.68	0.73	0.75	0.70	0.35
Phenylalanine	0.17	0.15	0.18	0.19	0.17	0.08
Threonine	0.21	0.24	0.27	0.29	0.26	0.05
Tryptophan	0.42	0.56	0.60	0.62	0.55	0.26
Valine	1.42	1.50	1.66	1.75	1.59	0.70
Alanine	0.23	0.30	0.33	0.35	0.30	0.08

T1= Raw MOS, T2= 1 Hour water-soaked MOS, T3= 2 Hours water-soaked MOS, T4= 3 Hours water-soaked MOS

MOS: *Moringa oleifera* seeds

SD: Standard Deviation

Table 16: Fatty Acid Profile of Raw and Water -Soaked *Moringa oleifera* Seed

Fatty acids (%)	T 1	T2	T3	T4	Mean	SD
Oleic	54.43	40.15	41.20	43.14	44.73	3.11
Behenic	7.00	6.92	6.54	6.85	6.83	0.75
Palmitic	6.50	5.38	5.52	5.88	5.82	0.62
Stearic	5.05	5.00	4.25	5.31	4.90	0.60
Linolenic	0.22	0.20	0.20	0.18	0.20	0.80
Myristic	0.46	0.51	0.50	0.53	0.50	0.16
Palmitoleic	2.00	2.23	2.30	2.20	2.18	0.09
Eicosenoic	1.80	1.90	1.75	1.95	1.85	0.76
Lignoceric	1.00	1.01	0.95	0.92	0.97	0.08
Arachidic	4.20	3.74	3.55	3.25	3.69	0.73

T1= Raw MOS, T2= 1 Hour water-soaked MOS, T3= 2 Hours water-soaked MOS, T4= 3 Hours water-soaked MOS

MOS: *Moringa oleifera* seeds

SD: Standard Deviation

4.7 Results on Performance of Albino Rats Fed *Moringa oleifera* Seed Meal

The relative effects of the various weaning feeds on the performance of albino rats are shown in Table 17.

4.7.1 Feed intake: The total feed intakes of rats were, 52.46, 40.26, 41.75 and 39.85g for dietary treatments 1, 2, 3 and 4, respectively. However, feed intake was significantly different (P>0.05) among treatments. Treatment 1 which is the control diet (casein) had the highest value, followed by treatment 3 which is water-soaked MOSM while treatment 4, the protein-free diet had the least value than others.

4.7.2 Weight Gain: The results on weight gain indicate that rats fed the control ratio (casein) had the highest body weight gain of 24.10g, followed by rats fed diets 3 and 2 with the values of 9.20 and 5.20g respectively. While rats fed the protein free diet had the least value of -11.30g. The differences in weight gain were significant (P>0.05) for all the dietary treatments.

4.7.3 Feed Conversion Ratio: The feed conversion ratio for the dietary treatments were significantly different (P<0.05) from the rats on control diet (casein) of the value of 2.14, while the dietary treatments had 4.54, 7.74 and -3.53 for diets 3, 2 and 4 respectively.

Table 17: Performance of Albino Rats Fed *Moringa oleifera* Seed

PARAMETERS	T1	T2	T3	T4	SEM
Initial Body weight(g) /day	42.80	41.40	41.70	44.20	1.35
Ave Final Body weight (g)/day	66.90[a]	45.40[b]	50.90[b]	32.38[c]	3.71
Ave Body Weight Gain (g)./day	24.10[a]	5.20[b]	9.20[b]	-11.30[c]	1.02
Ave Feed Intake (g)/day	51.46[a]	40.26[b]	41.75[b]	39.86[b]	5.41
Feed Conversion Ratio /rat	2.14[c]	7.74[a]	4.54[b]	-3.53[d]	0.49

[abcd] Means along same row with different superscripts are significantly different (P <0.05)

T1= Purified diet, T2= Raw MOS, T3= 3 Hours water-soaked MOS, T4=Protein free diet

MOS: *Moringa oleifera* seed

4.8: Table 18 shows the results on protein quality of Albino rats fed *Moringa oleifera* seed meal

4.8.1 Protein intake: Protein-intake followed the trend for feed intake and was significantly different (P>0.05) among the treatments with the values of 10.89, 8.54, 8.32 and 3.42g/day for diets 1, 3, 2 and 4, respectively. Rats fed the control diet had the highest value while rats fed treatment four (protein free diet) had the least value and T3 (water-soaked MOSM) and T2 (raw MOSM) were similar.

4.8.2 Net Protein Ratio (NPR): The results of the NPR were significantly different (P>0.05) for the various dietary treatments. Rats fed on diet 1 (Casein) had comparable NPR value with the dietary treatments, of 2.50, 1.33, 1.03 and -5.70% for diets 1, 2, 3 and 4, respectively.

4.8.3 Protein Retention Efficiency (PRE): The PRE values were significantly different (P>0.05) among treatments as rats fed casein diet (control) obtained the highest value of 40.10% while rats on raw (T2), water-soaked MOSM (T3) and the protein free diets (T4) obtained 21.35, 16.00 and 91.60%, respectively.

4.8.4 Biological Value (BV): Data on BV also showed a significant difference (P>0.05). The highest value was also recorded for rats fed on casein (90.91%) which was significantly higher than values obtained for rats fed other dietary treatments with the values of 65.69, 54.87, and 15.49% for raw (T2), water-soaked (T3) and protein free diet (T4), respectively.

4.8.5 Net Protein Utilisation (NPU): The results of the NPU showed that significant differences (P>0.05) existed between the dietary treatments. However, rats on the dietary treatments (15.86%) (44.37%) 2 and 3 had different results compared to the casein diet (52.70%) while the lowest NPU value was recorded among the rats fed on diet 4 (0.00%).

Table 18: Protein Quality of *Moringa oleifera* Seed Fed to Albino Rats

PARAMETERS (g)	T1	T2	T3	T4	SEM
Protein Intake	10.89[a]	8.32[b]	8.54[b]	3.42[c]	0.65
Feacal Nitrogen/	9.25[a]	5.80[b]	6.95[b]	2.10[c]	0.05
Urinary Nitrogen	13.20[a]	6.65[c]	9.55[b]	2.40[d]	0.08
Net Protein Ratio	2.50[a]	1.33[b]	1.03[b]	-5.70[c]	0.58
Protein Retention Efficiency	40.10[a]	21.35[b]	16.00[c]	-91.60[d]	2.08
Biological Value	90.91[a]	54.87[c]	65.69[b]	15.49[d]	1.65
Total Body Nitrogen	6.44[a]	2.02[c]	4.49[b]	0.70[d]	0.55
Total Body Water	22.97[a]	20.12[b]	18.45[bc]	15.16[c]	2.30
Net Protein Utilisation	52.70[a]	15.86[c]	44.37[b]	0.00	0.95
Protein Efficiency Ratio	2.21[a]	0.63[c]	1.10[b]	-3.30[d]	0.33

[abcd] Means along same row with different superscripts are significantly different (P <0.05)

T1= Purified diet, T2= Raw MOS, T3= 3 Hours water-soaked MOS, T4=Protein free diet

MOS: *Moringa oleifera* seed

4.9 Table 19 summarised the results of the haematological parameters obtained for albino rats fed raw and processed *Moringa oleifera* seed meal. There were significant ($P<0.05$) differences among the treatments as the values of packed cell volumes (PCV) ranges between 30.86-40.00% for rats fed protein-free diet (T4) and casein diet (T1), respectively. Rats on casein diet (T1) had the highest haemaglobin and red blood cell (RBC) of 13.3 and 7.62, respectively, which were significantly ($P<0.05$) higher than others, while rats on water-soaked MOSM (T3) had 12.19 for haemoglobin and 6.78 for RBC, but rats on protein free diet (T4) recorded the least values of 10.13 and 3.16 for haemoglobin and RBC, respectively.

The highest White Blood Cells (WBC) values (5.15) and WBC differntial counts were obtained in rat fed protein-free diet, which were significantly ($P<0.05$) higher than others, while rats on casein diet (T1), raw MOSM (T2) and water-soaked MOSM (T3) obtained the WBC values of 3.15, 3.30 and 4.65, respectively. There were no significant ($P>0.05$) differences among treatments in mean corpuscular volumes (MCV) and mean corpuscular haemoglobin concentration (MCHC) but there were differences ($P<0.05$) among treatments with MCH corpuscular haemoglobin (MCH) where rats on raw MOSM (T2) obtained the highest value of 35.71% followed by rats on casein diet (T1), and rats on water-soaked MOSM (T3) were intermediate (24.06%) and rats on protein-free diet (T4) had the least value (15.66%).

Table 19: Haematological Indices of Albino Rats Fed *Moringa oleifera* Seed Meal

PARAMETERS	T1	T2	T3	T4	SEM
Packed Cell Volume (%)	40.00[a]	33.33[b]	36.33[b]	30.86[c]	8.75
Haemoglobin(g/L)	13.33[a]	11.13[c]	12.19[b]	10.13[d]	2.12
Red Blood Cell (10^6/uL)	4.12[c]	3.16[d]	6.62[b]	7.78[a]	2.02
White Blood Cell (10^3/uL)	3.26[c]	3.00[c]	4.65[b]	5.15[a]	1.49
Lymphocytes (%)	66.33[b]	69.33[a]	58.00[c]	65.63[b]	8.31
Neutophils (%)	31.33[a]	25.00[b]	36.67[a]	29.33[ab]	0.38
Monocytes (%)	1.33[c]	3.33[a]	2.00[b]	2.67[ab]	0.50
Eosinophils (%)	1.00[c]	2.67[b]	3.33[a]	2.66[b]	0.15
MCV	400.00	333.67	363.33	363.33	18.75
MCH	32.57[a]	35.71[a]	24.06[b]	15.66[c]	0.87
MCHC	3.33	3.39	3.32	3.33	0.42

[abcd]Means along same row with different superscripts are significantly different (P <0.05)

T1= Purified diet, T2= Raw MOS, T3= 3 Hours water-soaked MOS, T4=Protein free diet

MOS: *Moringa oleifera* seed

4.10 The results of the serum biochemical variables are presented in table 20. There were significant (P<0.05) differences among the treatments. On the values obtained in total protein (TP), rats on casein diet (T1) had the highest value (5.73g/dL) which was significantly (P<0.05) different from other treatments, while others obtained 4.58, 4.69 and 2.92g/dl for raw MOSM (T2), water-soaked MOSM (T3) and protein-free diet (T4), respectively. There were no significant (P>0.05) different on the values of albumin. Rats on casein diet (T1) and protein-free diet (T4) had the highest cholesterol values (78.87 and 76.34mg/dl respectively) while rats on raw MOSM (T2) and water-soaked MOSM (T3) had the least values (70.09 and 68.65mg/dl, respectively). The values of urea ranges from 22.29mg/dL-43.64mg/dL for rats on protein-free diet (T4) and rats on casein diet (T1), respectively. The values obtained on Aspartate Amino Transferase (AST) and Alanine Transferase (ALT) were significantly (P<0.05) different among treatments. Rats on casein diet (T1) had the highest AST and ALT values of 218.45 and 24.33iu/L, respectively while rats on water-soaked MOSM had the least values of 163.80 and 18.79iu/L for AST and ALT, respectively. The values of rats on protein-free diets (T4) were similar.

4.11 Results on Histology of Albino Rats Fed *Moringa oleifera* Seed Meal

The results of histology of rats fed *Moringa oleifera* seed meal in table 21 revealed that there were significant differences (P<0.05) on both the kidneys and livers of the rats. There were mild congestion of the glomeruli and distension of the capillary vessels with numerous thromb in rats fed casein diet (T1), raw MOSM (T2) and protein free diets (T4) while rats fed water-soaked MOSM (T3) observed no vision lesion in their kidneys. However, the livers of rats fed water-soaked MOSM (T3) and rats on protein-free diet (T4), shared mild multiple foci of coagulative necrosis, while rats fed with casein diet (T1) and raw MOSM (T2) observed moderate congestion of the sinusoids and centrolobular veins, with slight coagulative necrosis of the hepatocytes.

Table 20: Serum Biochemical Variables of Albino Rats Fed *Moringa oleifera* Seed Meal

PARAMETERS	T1	T2	T3	T4	SEM
Total Protein(g/dL)	4.73[b]	4.58[b]	2.69[c]	5.92[a]	0.41
Albumin(g/dL)	0.47	0.36	0.49	0.45	0.04
Globumin(g/dL)	2.26[c]	4.22[b]	2.20[c]	5.47[a]	0.58
Cholesterol(mg/L)	60.87[b]	76.09[a]	70.65[ab]	79.34[a]	0.61
Urea(mg/dL)	23.64[b]	41.55[a]	42.29[a]	22.64[b]	6.01
AST(iu/L)	218.45[a]	193.00[c]	207.84[a]	162.47[b]	5.53
ALT(iu/L)	24.33[a]	18.03[b]	18.79[b]	12.97[c]	0.78

[abcd] Means along same row with different superscripts are significantly different (P <0.05)

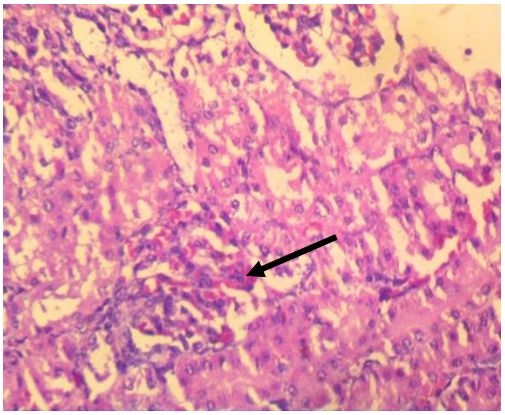
T1= Purified diet, T2= Raw MOS, T3= 3 Hours water-soaked MOS, T4=Protein free diet

MOS: *Moringa oleifera* seed

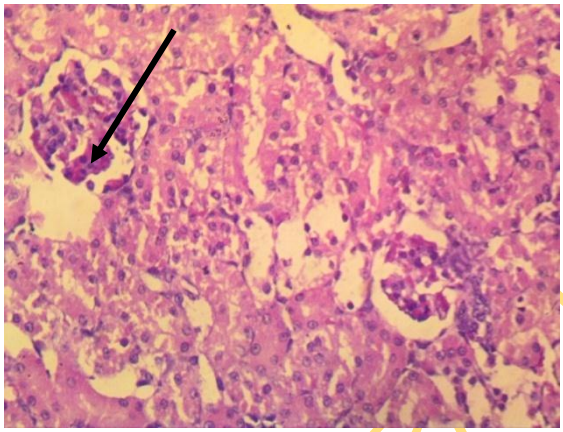
Table 21: Histology Assay of Albino Rats Fed *Moringa oleifera* Seed Meal

Parameters	T_1	T_2	T_3	T_4
Kidney (Necrosis/Lesion/Mild/Moderate/Severe)	Mild	Mild	NVL	Mild
Liver Necrosis/mild/moderate/severe)	Moderate	Moderate	Mild	Mild

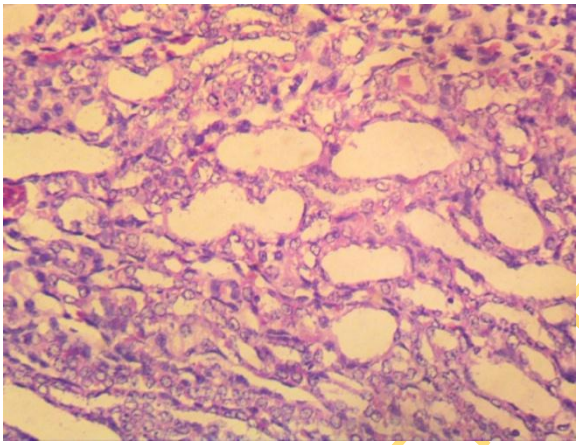
NVL: No visible lesion.



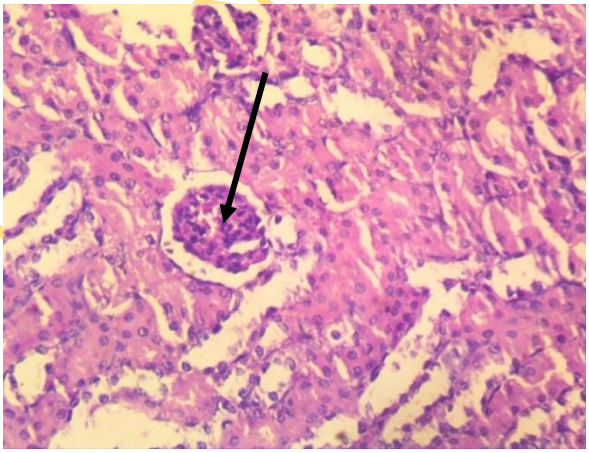
A: Histology of albino rat kidney fed casein diet (T1)



B: Histology of albino rat kidney fed raw MOSM (T2)



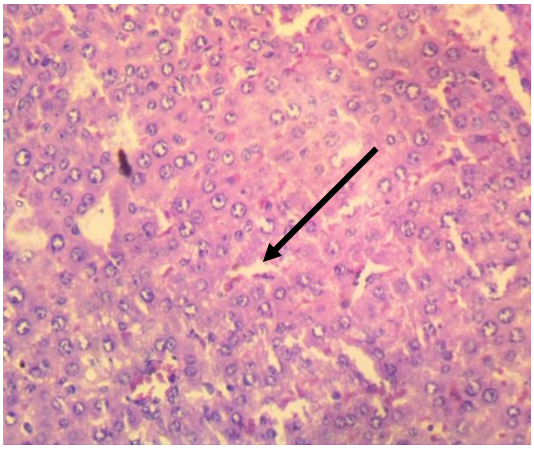
C: Histology of albino rat kidney fed water-soaked MOSM (T3



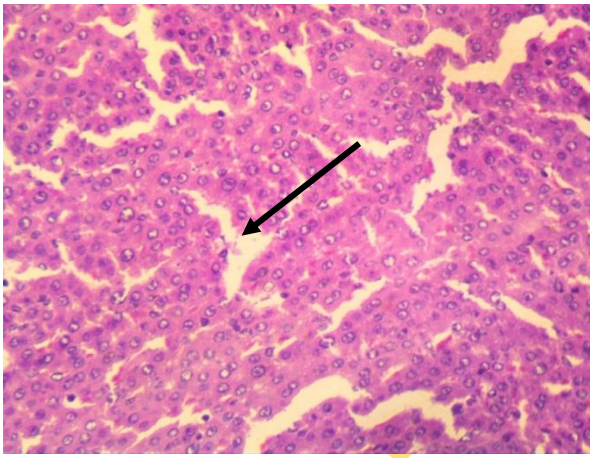
D: Histology of albino rat kidney fed protein free diet (T4)

(Mangification 400X)

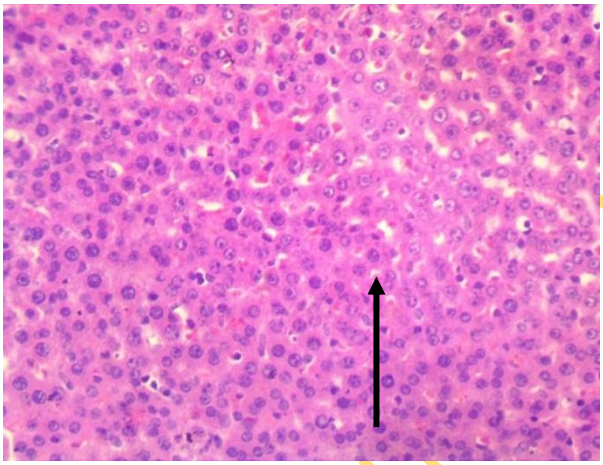
Plate 1: Histology of Albino Rat Kidney



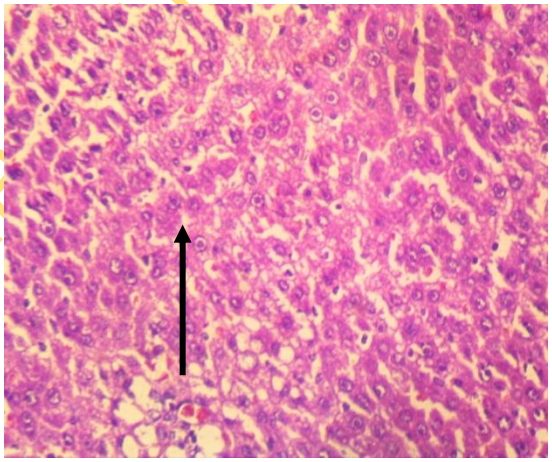
A: Histology of albino rat liver fed casein diet (T1)



B: Histology of albino rat liver fed raw MOSM (T2)



C: Histology of albino rat liver fed water-soaked MOSM (T3)



D: Histology of albino rat liver fed protein free diet (T4)

(Mangification 400X)

Plate 2: Histology of Albino Rat Liver

4.12 Proximate Composition of Feed Samples for Starter and Finishers Fed *Moringa oleifera* Seed Meal

The proximate composition of the text ingredients in tables 22 and 23 for starters and finishers, respectively, showed that *Moringa oleifera* seed meal significantly (P<0.05) increased the crude protein contents in the broiler starter feed. The values ranged between 15.40 and 16.20% for crude protein, 4.90 and 6.7% for crude fibre, 15.10 and 16.20% for total ash, 8.01 and 11.30% for ether extract and 45.90 and 55.20% for nitrogen free extract while the highest dry matter content was significantly higher (P<0.05) in diet on 25% MOS (81.77%) while 100% FFSB had the least value (73.12%). The values obtained for all samples were above 20%.

The proximate composition of feed samples in broilers finisher phase was significantly influenced (P<0.05) by the inclusion levels of *Moringa oleifera* seed. The crude protein content was significantly (P<0.05) higher (19.70%) in 100% MOS (T5) while no differences occurred between 100% FFSB (15.75%) and 50% MOS (15.05%). The crude fibre contents increased with the inclusion levels of Moringa seeds where values ranged between 3.14 and 8.94%. The total ash contents ranged between 6.82 and 10.96%, ether extracts ranged between 15.54 and 17.22% while the nitrogen free extracts ranged between 47.32 and 57.50%. The moisture contents for all samples were below 10% (90% dry matter).

4.13 Performance Characteristics for Broiler Starters and Finishers Fed *Moringa oleifera* Seed Meal

The performance of starter and finisher broilers fed *Moringa oleifera* seed meal are presented in Table 24 and Table 25, respectively. There was feed depression as the levels of moringa increased, There were significant differences (p<0.05) in final weight, body weight gain and feed conversion ratio of birds among treatments. Birds fed on T1 (control) recorded significantly (p<0.05) the highest body weight gain and total feed intake compare to other treatments. Birds on T5 (100% MOSM) had the lowest body weight, body weight gain and feed intake, but had the highest feed conversion ratio than other treatments

Table 22: Proximate compositions of Feed Samples for Broiler Starters Fed *Moringa oleifera* Seed Meal

Parameters (%)	T_1	T_2	T_3	T_4	T_5
Dry matter	73.12	81.77	74.61	80.57	74.66
Crude protein	21.70	15.75	19.95	15.40	25.20
Crude fibre	4.90	6.00	6.10	5.50	6.70
Ash	16.20	16.01	15.54	15.90	15.10
Ether Extract	10.20	11.30	10.54	8.01	9.11
NFE	46.00	50.90	47.90	55.20	45.90

Table 23: Proximate Compositions of Feed Samples for Broiler Finishers Fed *Moringa oleifera* Seed Meal

Parameters (%)	T_1	T_2	T_3	T_4	T_5
Crude protein	15.75	17.85	15.05	16.35	19.70
Crude fibre	3.90	3.14	4.40	6.10	8.94
Ash	8.40	10.96	7.59	10.01	6.82
Ether Extract	15.54	16.01	15.41	17.22	16.83
Dry matter	95.90	94.50	96.10	95.04	94.93
NFE	56.40	52.00	57.50	47.32	52.71

Table 24: Performance Characteristics of Broiler Chickens Fed *Moringa oleifera* Seed Meal at Starter Phase

Parameters	T_1 (control)	T_2 (25%)	T_3 (50%)	T_4 (75%)	T_5 (100%)	SEM
Initial Body Weight (g/bird)	210.42	200.65	205.11	207.85	201.56	2.47
Final Body Weight (g/bird)	783.48^{a}	700.41^{a}	682.66^{b}	654.30^{b}	552.19^{c}	39.83
Weight Gain (g/bird)	573.06^{a}	499.76 ^{b}	477.55^{b}	446.45^{c}	350.63^{d}	28.97
Total Feed Intake (g/bird)	1485.00^{a}	1413.42^{a}	1386.50^{ab}	1300.85^{b}	1290.62^{b}	50.75
Feed conversion ratio	2.60^{b}	2.83^{b}	2.90^{b}	2.91^{b}	3.69^{a}	0.20

^{abc} Means along same row with different superscripts are significantly different (P <0.05)

Table 25: Performance Characteristics of Broiler Chickens Fed *Moringa oleifera* Seed Meal at Finisher Phase

Parameters	T_1 (control)	T_2 (25%)	T_3 (50%)	T_4 (75%)	T_5 (100%)	SEM
Initial Body Weight (g/bird)	783.48[a]	700.41[a]	682.66[b]	654.30[b]	552.19[c]	39.83
Final Body Weight (g/bird	2008.66[a]	1709.71[b]	1672.27[b]	1500.25[c]	1398.14[d]	45.37
Weight Gain (g/bird)	1225.18[a]	1009.30[b]	989.61[c]	845.95[d]	631.79[e]	83.16
Total Feed Intake (g/bird)	3000.00[a]	2850.13[b]	2625.85[b]	2470.21[c]	2198.63[d]	83.16
Feed conversion ratio	2.45[c]	2.83[b]	2.65[b]	2.92[b]	3.48[a]	0.12

[abcde]Means along same row with different superscripts are significantly different (P <0.05)

4.14 The Nutrient Digestibility of Broiler Chickens Fed *Moringa oleifera* Seed Meal

Results on nutrient digestibility presented in Table 26 revealed that there were significant differences observed as birds on T5 (100% MOSM) had the highest nutrient digestibility values of 85.65, 31.53, 46.12(%), for crude protein, crude fibre and ether extract, respectively, while T1 (control) had the highest dry matter content. Among birds on the test diets, birds on treatment five (100% MOSM) had a better mean crude protein digestibility value which was significantly different ($p<0.05$) than those on other diets.

4.15 Haematological Indices of Broiler Chickens Fed *Moringa oleifera* Seed Meal

The haematological indices are presented in table 27. It was observed that birds fed the highest inclusion level of MOSM (T5) had the highest values of Pack Cell Volume (PCV) and haemoglobins (H) with the values of 35.50 and 11.77% respectively while T1 (control) and T2 (25% MOSM) obtained the lowest values of 29.67, 29.33 and 9.16, 9.73% respectively. There were no significant differences ($p>0.05$) in the values obtained for Red Blood Cells (RBC), Eosinophils and Basophils among the treatments. T1 (control) obtained the highest value on white blood cells (WBC) 1.52 x 10^3 while T4 (75% MOSM) had the least value of 1.24 x 10^3.

4.16 Serum Biochemical Variables of Broiler Chickens Fed *Moringa oleifera* Seed Meal

The results of serum biochemical variables are presented in Table 28. There were significant differences ($p<0.05$) among treatments, in some of the serum biochemical variables except for total protein, globulin and ALP which has no significant differences ($p>0.05$) among treatments. T1 (control) obtained the highest values on Albumin, AST, ALT, Uric acid, creatinine and glucose which was significantly higher than others with the values of 2.03(g/dL), 273.67 (iu/L), 37.00 (iu/L), 18.21 (mg/dL), 1.05 (mg/dL) and 324.05 (mg/dL), respectively, while T5 (100% MOSM) had the least values on Albumin (1-13(g/dl), AST (196.09 (iu/l), uric acid (13.67 (mg/dl), creatinine (0.60 (mg/dl) and glucose (218.92(mg/dl), and T2 (25% MOSM), T3 (50% MOSM) and T4 (75% MOSM) were at the middle.

Table 26: Nutrient Digestibility of Broiler Chickens Fed *Moringa oleifera* Seed Meal

Parameters (%)	T_1	T_2	T_3	T_4	T_5	SEM
Crude protein	60.22^{c}	77.60^{b}	79.86^{b}	69.94^{b}	85.65^{a}	2.51
Dry matter	33.12^{a}	21.60^{b}	28.13^{ab}	21.49^{b}	21.92^{b}	4.08
Crude fibre	25.02^{b}	26.47^{b}	30.84^{a}	28.43^{b}	31.53^{a}	0.81
Ether Extract	34.35^{b}	29.67^{c}	30.13^{b}	38.12^{b}	46.12^{a}	1.76
Nitrogen Free Extracts	19.59	33.74	40.83	36.49	63.30	7.62

abc. Means along same row with different superscripts are significantly different (P <0.05)

Table 27: Haematological Indices of Broiler Chickens Fed *Moringa oleifera* Seed Meal

Parameters	**$T_{1\ (control)}$**	**$T_{2\ (25\%)}$**	**$T_{3\ (50\%)}$**	**$T_{4\ (75\%)}$**	**$T_{5\ (100\%)}$**	**SEM**
Packed cell volume (%)	29.67^{b}	29.33^{b}	32.38^{a}	34.67^{a}	34.50^{a}	0.86
Hemoglobin (g/100ml)	9.16^{c}	9.73^{c}	10.60^{b}	11.56^{a}	11.77^{a}	0.31
Red blood cell ($x10^{6}$/l)	3.28^{a}	2.98^{b}	3.33^{a}	3.44^{a}	3.42^{a}	0.08
White blood cell ($x10^{3}$/l)	1.53^{a}	1.34^{b}	1.35^{b}	1.25^{c}	1.38^{b}	0.52
Platelets ($x10^{5}$/l)	1.29^{a}	1.25^{a}	1.01^{c}	1.18^{b}	1.04^{c}	0.08
Lymphocytes (%)	60.35^{b}	62.50^{b}	69.55^{b}	74.00^{a}	68.52^{b}	1.59
Heterophils (%)	32.33^{a}	29.67^{ab}	23.33^{b}	18.41^{c}	24.67^{b}	1.65
Hetrophils-Lymphocyte Ratio	0.54^{a}	0.47^{a}	0.36^{b}	0.25^{c}	0.36^{b}	0.06
Monocytes (%)	4.00^{a}	3.67^{b}	2.66^{c}	3.67^{b}	3.67^{b}	0.26
Eosinophils (%)	3.00	3.67	4.33	3.67	3.00	0.32
Basophils (%)	0.33	0.33	0.33	0.33	0.30	0.12

^{abc} Means along same row with different superscripts are significantly different (P<0.05)

Table 28: Serum Biochemical Variables of Broiler Chickens Fed *Moringa oleifera* Seed Meal

Parameters	$T_{1\ (control)}$	$T_{2\ (25\%)}$	$T_{3\ (50\%)}$	$T_{4\ (75\%)}$	$T_{5\ (100\%)}$	SEM
Albumin (g/dL)	2.03^{a}	1.13^{c}	1.46^{b}	1.38^{b}	1.17^{c}	0.18
Globulin (g/dL)	3.17	3.43	3.06	3.33	3.30	0.11
Albumin-Globulin ratio	0.60^{a}	0.33^{c}	0.48^{b}	0.33^{c}	0.33^{c}	0.07
AST (iu/L)	213.67^{a}	196.50^{b}	232.41^{a}	200.50^{b}	198.09^{b}	9.40
ALT (iu/L)	32.00^{a}	36.50^{a}	35.33^{a}	25.56^{b}	37.50^{a}	2.42
ALP (iu/L)	386.31	391.67	386.22	386.40	354.33	11.96
Uric Acid (mg/dL)	18.21	17.33	16.95	18.30	17.67	0.96
Creatinine (mg/dL)	1.05^{a}	0.60^{b}	0.67^{b}	0.60^{b}	0.66^{b}	0.06
Glucose (mg/dL)	284.05	292.10	264.18	258.00	238.92	13.95

^{abc} Means along same row with different superscripts are significantly different (P<0.05)

AST, Aspatate amino transferase, ALT, Alanine amino transferase, ALP, Alanine phosphate

4.17 Lipid Profile of Broiler Chickens Fed *Moringa* Seed Meal

Table 29; presented the results of lipid profile (total cholesterol, triglyceride, high density and low density liproproteins) of broiler chickens fed MOSM. From these results, it was observed that values on the dietary treatments were significantly lower ($p>0.05$) than the control. Total cholesterol values ranges between 157 and 206(mg/dL) where the highest cholesterol concentration occurred in 100%FFSB and lowest in 100%MOSM. Triglyceride values were higher in 100%FFSB (85.36mg/dL) and lower in 100%MOSM (56.00mg/dL).Values of high density lipoprotein ranged between 90.30 and 120.51mg/dL, while low density lipoprotein values were higher in 75%MOSM (89.07mg/dL) and lower in 100%MOSM (64.80mg/dL).

4.18 Immunoglobulin Assay of Broiler Chickens Fed *Moringa oleifera* Seed Meal

Results on Table 30, shows the immunoglobulin assay of broiler chickens fed MOSM. From these results it was observed that there were significant differences ($p<0.05$) among the moringa treatments compared to the control diet. The higher the concentration of MOSM, the higher the values obtained from the immunoglobins. .The values of immunoglobulin IgG ranged between 0.59 and 0.73(iu/dL) where the highest concentration was observed in 100%MOSM. The IgA values ranged between 0.26 and 0.49(iu/dl) where 25%MOSM obtained the least and 100%MOSM had the highest immunoglobulin values. Also IgM values were also higher in 100%MOSM (0.15iu/dL) while 100%FFSB had the least value (0.09iu/Dl).

4.19 Carcass Characteristics Fed *Moringa oleifera* Seed Meal

In table 31, there were significant relationship between the carcass characteristics and the feed offered, because the parameters on carcass characteristics reduced as the inclusion level of *Moringa oleifera* seed also increased, where values ranged between 1.41 and 2.87g for final weight, 1.13 and 1.30g for de-feathered weights, 0.69 and 1.03g for eviscerated weight. The dressing percentage was significantly lower ($P<0.05$) in 100% FFSB with the value of 35.20% and higher in 25% MOSM with 58.19% value.

Table 29: Lipid Profile of Broiler Chickens Fed *Moringa oleifera* Seed Meal

Parameters(mg/dl)	T_1	T_2	T_3	T_4	T_5	SEM
Total cholesterol	206.33[a]	196.67[a]	169.52[b]	164.50[b]	157.67[b]	9.32
Triglycerides	85.36[a]	82.77[a]	63.61[b]	76.33[ab]	56.00[c]	8.26
High density lipoprotein	102.37[b]	110.00[b]	120.51[a]	90.30[c]	101.69[b]	5.56
Low density lipoprotein	86.60[a]	83.47[a]	86.27[a]	89.07[a]	64.80[b]	8.29

[abc] Means along same row with different superscripts are significantly different (P<0.05)

Table 30: Immunoglobulin Assay of Broiler Chickens Fed *Moringa oleifera* Seed Meal

Parameters (iu/dl)	T_1	T_2	T_3	T_4	T_5	**SEM**
1gG	0.62^{ab}	0.59^{b}	0.65^{ab}	0.62^{ab}	0.73^{a}	0.06
1GA	0.311^{b}	0.26^{b}	0.37^{ab}	0.42^{a}	0.49^{a}	0.08
1GM	0.09^{b}	0.12^{ab}	0.14^{a}	0.14^{a}	0.15^{a}	0.08

^{ab} Means along same row with different superscripts are significantly different (P<0.05)

Table 31: Carcass Characteristics of Broiler Chickens Fed *Moringa oleifera* Seed Meal

Parameters	T_1	T_2	T_3	T_4	T_5	SEM
Initial weight (g/bird)	210.42	200.65	205.11	207.85	201.56	2.31
Final weight (g/bird)	2.87[a]	1.77[b]	1.73[b]	1.53[c]	1.41[d]	0.82
Bled weight(g/bird)	1.45[a]	1.33[b]	1.22[c]	1.27[c]	1.30[b]	0.45
De-feathered wt (g/bird)	1.22[c]	1.30[a]	1.22[c]	1.24[b]	1.13[d]	0.49
Eviscerated wt (g/bird)	1.01[a]	1.03[a]	0.85[b]	0.87[b]	0.69[c]	0.25
Dressing (%)	35.20[c]	58.19[a]	49.13[b]	56.86[a]	48.93[b]	3.63

[abcd] Means along same row with different superscripts are significantly different (P<0.05)

4.20 Histological Assay of Broiler Chickens Fed *Moringa oleifera* Seed Meal

The results of histology of broiler chickens fed *Moringa oleifera* seed meal in Table 32 showed the different levels of damage done to the different organs by the different treatments at different inclusion levels. The degree of damage done by the diets to the organs is rated as follows; mild, moderate and severe. In the heart, it was observed that T1 (control), T3 (50% MOSM), T4 (75% MOSM) and T5 (100.0% MOSM) had normal cardiomyocytes in their hearts while T2 (25% MOSM) had few or mild foci of necrosis of cardiomyocytes in their hearts.

4.21 Primal Cuts of Broiler Chickens Fed *Moringa oleifera* Seed Meal

Table 33 showed the results of the primal cuts of broiler chickens fed MOSM which revealed that birds on complete FFSB (T1) obtained the highest values in all the cut parts which was significantly different ($p<0.05$) from other treatments. The relationship among treatment groups live weight and cut parts changed significantly as the increase in the concentration of *Moringa* seed meal. The relative weight of chest, wings, thigh, drumstick and back of birds fed the control diets were significantly higher ($p<0.05$) while birds fed 100% MOSM (T5) had the least values, this could be as a result of the final weights and carcass weights

4.22 Organ Characteristics of Broiler Chickens Fed *Moringa oleifera* Seed Meal

Table 34 shows the organ characteristics of bird fed moringa seed meal. The treatments significantly ($P<0.05$) influenced the organs of broiler chickens fed moringa and full-fat soyabean. The relative heart weight was significantly higher ($P<0.05$) in birds fed 100.0% MOS (100.0% MOS (0.56%) while the lower value was obtained in birds fed 100% FFSB (0.20%). The values of liver ranged between 1.85 and 2.42%, lungs ranged between 0.35 and 0.60%, kidney ranged between 0.37 and 0.46%, proventiculus ranged between 0.26 and 0.40% while the highest spleen value was obtained in 100.0% MOS and lowest value obtained in 100.0% FFSB with the values of 0.86 and 0.56% respectively. The intestine weight was higher in 50.0% MOS and 100.0% MOS with 0.14% values for each and birds on 100.0% FFSB had the least value (0.09%). The highest gall bladder weight was lower in birds fed 100.0% FFSB (2.61%) and higher in birds fed 100.0% MOS (7.70%), while birds on 75.0% MOS had the least gizzard value

(0.06%) and birds on 100% MOS had the highest value (0.14g), but for abdominal fat pad, birds on 100% FFSB (T1) had the highest value which was significantly difference ($p<0.05$) from the moringa treatments with the value of 4.83 compared to 2.97% in birds fed 100% MOSM.

4.23 Proximate Composition of Meat Samples of Broiler Chickens Fed *Moringa oleifera* Seed Meal

The proximate composition of the meat of broiler chickens fed Moringa seed meal in table 35 showed that there were significant differences ($p<0.05$) among the treatments as the control diet obtained the highest values on dry matter and ether extract with the values of 36.51 and 13.68% respectively, while T5 (100% MOSM) had the least values of 29.45% and 3.53%, respectively. For the crude protein and Ash, T5 (100% MOSM) had the highest values of 24.58 and 1.75%, respectively while T2 (25% MOSM) T3 (50% MOSM) and T4 (75% MOSM) were at the middle.

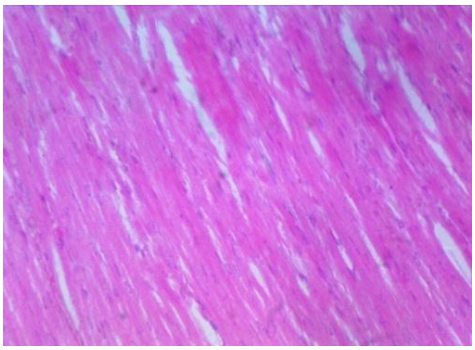
4.24 Sensory Evaluation of Meat Samples of Broiler Chickens Fed *Moringa oleifera* Seed Meal

The sensory evaluation of broiler chickens fed *Moringa oleifera* seed meal are shown in table 36. As the inclusion level of *Moringa* seed meal increased, the quality of the meat also reduced. Except on the meat colour, which was noticed that T2 (25% MOSM) and T5 (100% MOSM) had the highest values of 7.67 and 7.00, respectively. T3 (50% MOSM) and T5 (100% MOSM) had the highest aroma values of 5.33 and 5.33, respectively.

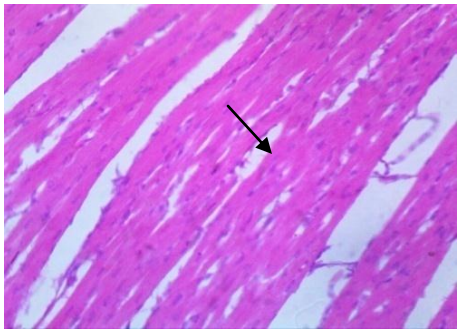
Table 32: Histology Assay of Broiler Chickens Fed *Moriinga oleifera* Seed Meal

Parameters	T_1	T_2	T_3	T_4	T_5
Heart (Necrosis/lesion Moderate/mild severe	NVL	Mild	NVL	NVL	NVL
Kidney (Necrosis/Lesion Mild/moderate/severe	Severe	Severe	Mild	Severe	Mild
Liver (Necrosis/Lesion, Moderate/mild/severe	Mild	Moderate	Moderate	NVL	NVL

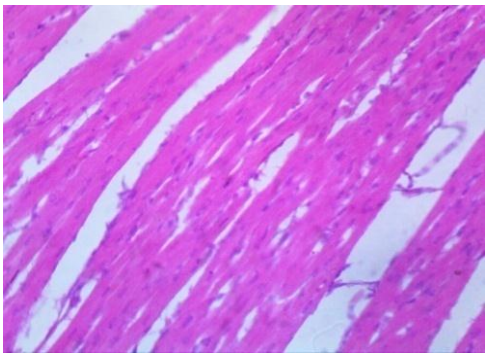
NVL: No visible Lesion



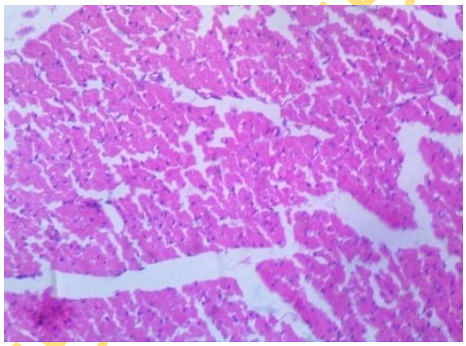
A: Histology of broiler chicken heart fed 100% FFSB (T1)



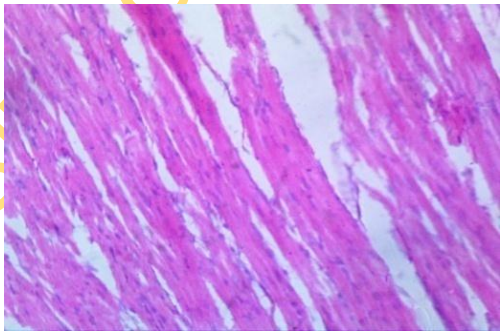
B: Histology of broiler chicken heart fed 25% MOSM (T2)



C: Histology of broiler chicken heart fed 50% MOSM (T3)



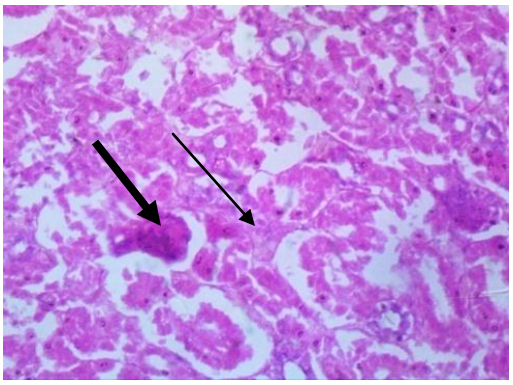
D: Histology of broiler chicken heart fed 75% MOSM (T4)



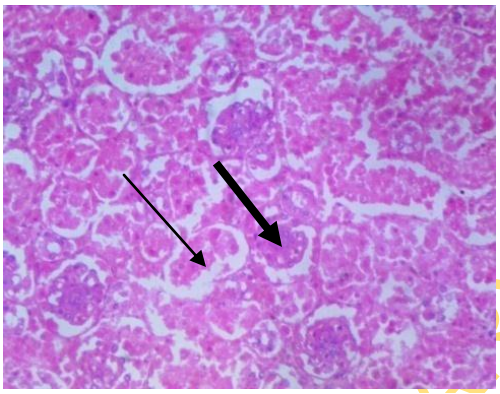
E: Histology of broiler chicken heart fed 100% MOSM (T5)

(Mangification 400X)

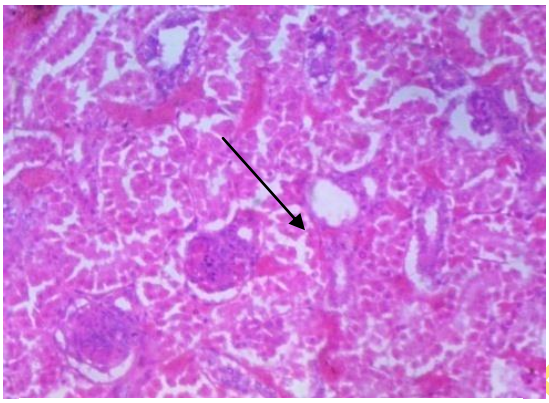
Plate 3: Histology of Broiler Chicken Heart



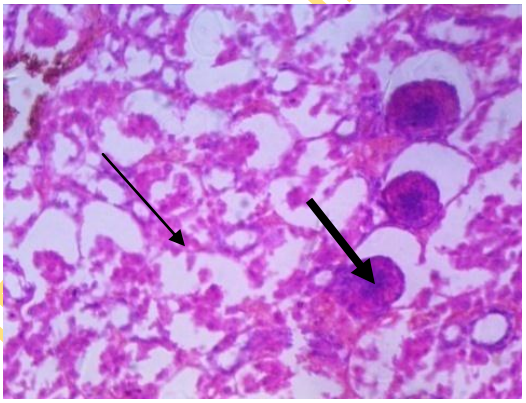
A: Histology of broiler chicken kidney fed 100% FFSB (T1)



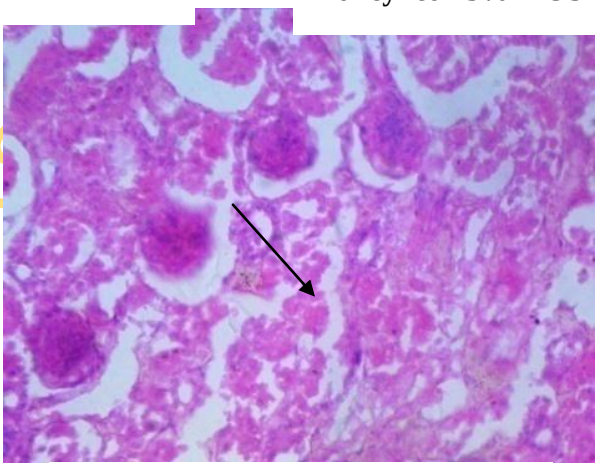
B: Histology of broiler chicken kidney fed 25% MOSM (T2)



C: Histology of broiler chicken kidney fed 50% MOSM (T3)



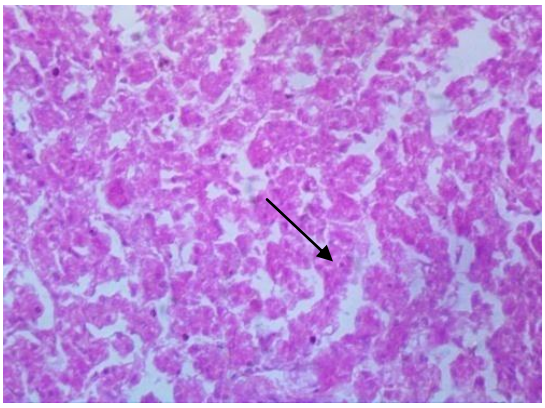
D: Histology of broiler chicken kidney fed 75% MOSM (T4)



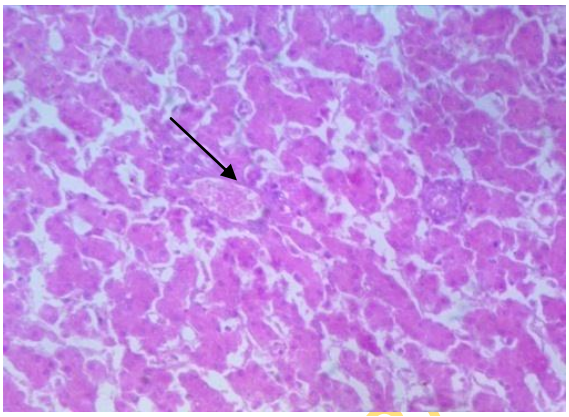
E: Histology of broiler chicken kidney fed 100% MOSM (T5)

(Mangification 400X)

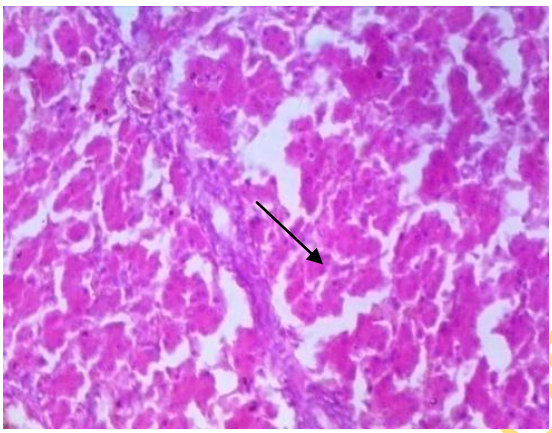
Plate 4: Histology of Broiler Chicken Kidney



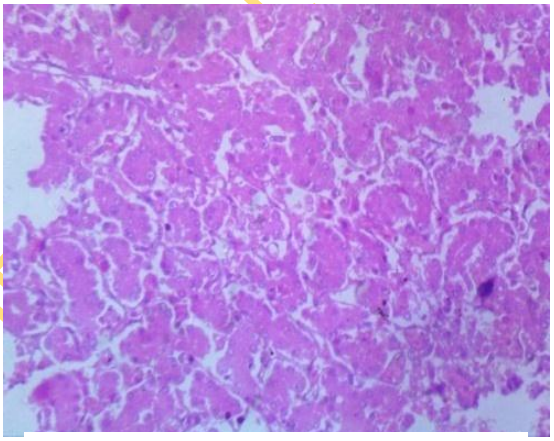
A: Histology of broiler chicken liver fed 100% FFSB (T1)



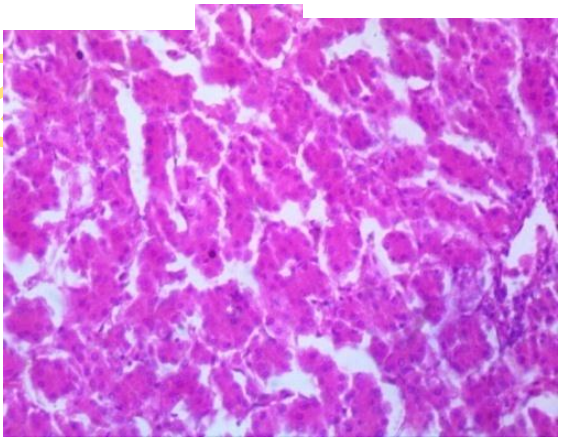
B: Histology of broiler chicken liver fed 25% MOSM (T2)



C: Histology of broiler chicken liver fed 50% MOSM (T3)



D: Histology of broiler chicken liver fed 75% MOSM (T4)



E: Histology of broiler chicken liver fed 100% MOSM (T5)

(Mangification 400X)

Plate 5: Histology of Broiler Chicken Liver

Table 33: Relative Weights of Primal Cuts of Broiler Chickens fed *Moringa oleifera* Seed Meal (g/100g body weight)

Parameters (%)	T_1 (Control)	T_2 (25%)	T_3 (50%)	T_4 (75%)	T_5 (100%)	SEM
Breast	14.77^{a}	14.02^{a}	13.78^{b}	12.99^{c}	11.77^{d}	0.23
Wings	7.11^{a}	7.02^{a}	$6..20^{b}$	6.93^{ab}	6.10^{c}	0.61
Thigh	8.75^{a}	8.64^{a}	7.66^{c}	8.10^{b}	7.20^{d}	0.32
Back	10.25^{a}	9.78^{b}	7.80^{d}	8.23^{c}	7.22^{e}	0.47
Drumstick	8.36^{a}	8.25^{a}	6.96^{c}	7.23^{b}	7.22^{b}	0.34

^{abcde} Means along same row with different superscripts are significantly different (P<0.05)

Table 34: Relative Weights Organs of Broilers Chickens Fed *Moringa oleifera* Seed Meal

Parameters (%)	T_1 (Control)	T_2 (25%)	T_3 50%	T_4 75%	T_5 100%	SEM
Heart	0.20^{d}	0.39^{c}	0.44^{b}	0.54^{a}	0.56^{a}	0.35
Liver	1.85^{c}	2.01^{b}	1.98^{c}	2.10^{b}	2.42^{a}	0.04
Lungs	0.41^{b}	0.42^{b}	0.35^{c}	0.41^{b}	0.60^{a}	0.06
Kidneys	0.39^{b}	0.40^{ab}	0.37^{b}	0.46^{a}	0.45^{a}	0.03
Proventiculus	0.26^{c}	0.30^{b}	0.32^{b}	0.36^{ab}	0.40^{a}	0.03
Spleen	0.56^{c}	0.64^{b}	0.62^{b}	0.68^{b}	0.86^{a}	0.05
Intestine	0.09^{c}	0.11^{b}	0.14^{a}	0.12^{b}	0.14^{a}	0.02
Gall bladder	2.61^{e}	7.26^{b}	5.82^{d}	6.66^{c}	7.70^{a}	1.07
Gizzard	0.08^{c}	0.10^{b}	0.08^{c}	0.06^{d}	0.14^{a}	0.01
Abdominal fat pad	4.83^{a}	3.39^{b}	3.76^{b}	3.51^{b}	2.97^{c}	0.16

^{abcd} Means along same row with different superscripts are significantly different (P<0.05)

Table 35: Proximate Compositions of Broiler Meat Samples Fed *Moringa oleifera* Seed Meal

Parameters (%)	T_1	T_2	T_3	T_4	T_5	SEM
Crude protein	20.73^{d}	22.48^{b}	21.39^{c}	24.31^{a}	24.58^{a}	0.41
Ash	1.43^{c}	1.42^{c}	1.61^{b}	1.31^{d}	1.71^{a}	0.14
Ether Extract	13.68^{a}	10.62^{b}	8.55^{c}	7.17^{d}	3.53^{e}	0.91
Dry matter	36.51^{a}	35.39^{b}	32.86^{c}	31.43^{d}	29.45^{e}	0.69
Nitrogen free extract	64.16^{d}	65.48^{c}	68.45^{b}	67.21^{b}	70.18^{a}	1.50

^{abcde} Means along same row with different superscripts are significantly different (P<0.05)

Table 36: Sensory Evaluation of Broiler Chickens Fed *Moringa oleifera* Seed Meal

Parameters	**T_1**	**T_2**	**T_3**	**T_4**
Aroma	4.33	3.17	5.33	3.33
Colour	6.33	7.67	6.00	6.00
Flavour	5.00	2.67	4.00	3.35
Tenderness	6.67	6.00	4.65	5.67
Juiceness	5.65	3.00	2.65	3.00
Overall acceptability	6.67	5.00	5.00	5.33

CHAPTER FIVE

DISCUSSION

5.1 Proximate Compositions of *Moringa oleifera* Seed

The result obtained in this study suggests a fairly high concentration of proximate components in the processed *M. oleifera* seed. As the soaking time increased, the values of crude protein, and crude fibre also increased, this is in line with Makkar and Becker (1997) who recommended that *M. oleifera* seeds soaked in water showed increased crude protein value. This increment will be as a result of a possible fermentation that took place during three hours of soaking and 3 days sun drying which is also in line with Ologhobo *et al.* (2006) who reported increment in crude fibre on processed lima bean varieties. Increasing soaking time decreased ether extract, dry matter and ash, which implies that the soaking hours had effect on the *M. oleifera* seed which is in line with Makkar and Becker (1997).

5.2 Mineral Compositions of *Moringa oleifera* Seed

The calcium contents, ranging between 0.42 and 0.44mg/100 were comparatively low in one-hour water-soaked MOS than two-hour water-soaked MOS. Calcium has been known to be necessary for bone and teeth formation. Hence it is very necessary for young chicks. The Moringa seed that was used in this study contained appreciable amount of calcium which compared well with those of other authors. In this study, calcium values obtained were higher than those reported by Mutayoba *et al*. (2011) and Ogbe and Affiku (2012) who reported calcium values of 0.25mg/100 and 0.38mg/100 respectively on *Moringa oleifera* leaf meal. Deficiency of calcium in poultry nutrition can affect their performance and health.

The level of phosphorus contained in the raw Moringa seed (T_1) (0.40mg/100) is slightly higher than the water-soaked Moringa seeds. Phosphorus values in this study are lower than reported by

Makkar and Becker (2009) (0.71mg/100), and Ogbe Affiku (2012) (0.9/mg/100). The low phosphorus values obtained in this study could be as a result of presence of phytic acid which reduce the nutritive value of legumes with respect to phosphorus and other essential minerals. The nutritional importance of phytic acid, lies in its ability to chelate several mineral elements especially phosphorus, thereby reduce their availability in the intestinal tract (Ogbe 2009). Ologhobo *et al*. (2006) found that phytic acid reduced the absorption of phosphorus and responsible for the rachitogenic properties in some cowpea legumes.

The most abundant element in *Moringa oleifera* seeds obtained in this study was potassium with concentration ranges of 0.92-0.95(mg/100). Adequate intake of Moringa seed could thus provide sufficient potassium to meet the needs of both animals and humans and thereby avert the danger of muscular weakness and paralysis often associated with low levels of plasma potassium. The plant seeds used in this study could also used to offset losses in body potassium reported to be common in kwashiorkor rats (Makkar and Becker 2007). Ogbe Affiku (2012) reported 0.97(mg/100) potassium in raw *Moringa oleifera* seed, which is in line with the values obtained in this study. They reported that high level of potassium in a legume diet can reduce the risk of hypertension when fed to rats and as well prevent dehydration and muscular cramp.

Magnesium plays a significant role in energy production and in supporting the immune system (Mohammed *et al.*, 2011). It also works with vitamin K to support blood clotting and with vitamin B complex to control the effects of stress (Sola-Ojo *et al*., 2013). The values of magnesium obtained in this study compared favourably with those reported by other authors. Ogbe and Affiku (2012) (0.58mg/100) and Makkar and Beckeer (2009) (0.52mg/100), the values in this study ranges from 0.42-0.54(mg/100).

The iron values obtained in this study are within the requirement range stipulated for all classes of animals. Iron requirement has been estimated by NRC (1994) and summed up on daily basis, for pigs 35mg, 45mg daily for calves, 0.45mg for rats and for chicks 30mg daily. More workers have used between 0.25 to 0.35mg per day for rats and got satisfactory results (Makkar and Becker (2009). Since the values of iron in this study are adequate in meeting the needs of the aforementioned classes of animals, their availability, utilisation and factors affecting them are

worth nothing. The ascorbic acid and lysine contents of Moringa seeds can help in the reduction of the ferric iron to the ferrous state and according to Mohammed and Zakanya (2011), ferrous iron salt is better utilised for haemoglobin formation than the ferric iron. Mutayoba *et al*. (2011) have attributed low availability of iron to a protein with a high affinity.

In summary, mineral content in plant seed is dependent on the type of plant and soil type supporting the growth of such plants, indicating that while comparisons are made of mineral levels in plant products, such comparisons should be based on relative averages and should themselves be held in loose esteem. Deficiency of these minerals are known to affect the performance and health of poultry, but *Moringa oleifera* seed is high in these minerals analysed (Kakengi *et al*., 2007).

5.3 Phytochemical Contents of *Moringa oleifera* Seed

Among the different soaking hour methods studied. Three hours of soaking in water was effective in eliminating anti nutritional factors of *M. oleifera* Seed. Such water-soaked destruction has been reported for several seeds including Jack bean (Babar, 1988), soyabean (Batra *et al.*, 1996), lima bean (Ologhobo *et al*., 2006), *Moringa oleifera* (Makkar and Becker, 2009), Jack bean (Amusa, 2011). Observations on *alkaloids* and *Saponins*, however, indicated significantly decreased in values on the dietary treatment than the control. The *phytic acid* content of *Moringa oleifera* seed was reduced only in 3 hours water-soaked. Liu and Makkar (2007) had attributed the decreased in *phytic acid* loss on soaking in water for 48 hours to the complexing of inositol hexaphosphate with Ca and Mg to form insoluble phytates which could be extracted only with dilute acids. This possibly explains the decrease in *phytic acid* loss when *Moringa oleifera* seed was soaked in water. *Cyanogens* and *Glucosinolates* values were also decreased significantly as the soaking time increases; this would be consistent with the observation with respect to lima bean where hydrogen cyanogens were reduced with different processing methods (Ologhobo *et al.,* 2006; and Makkar and Becker, 2009). There were no significant differences on the values of phenols but numerically there were slightly reductions in treatments 3 and 4 which indicate that *tannic* acid however, must have been lost through increased hours of soaking of a small fraction of hydrolysable *phenolic* compounds located in the seed coats of *M. oleifera.* Some amount of polyphenols have been found in soaking and cooking

waters of *M. oleifera* seed, indicating that large amounts of polyphenols could be eliminated by discarding washing and cooking waters (Makkar and Becker, 2009 and Liener, 2005).

5.4 Fibre Fraction Determinations of *Moringa oleifera* Seed

In digestibility work, a knowledge of the actual composition of the fibre would make for increased precision. Under certain circumstances, sufficient information might be obtained from an approximate figure for cellulose + lignin and cellulose + hemicelluloses together with the actual lignin content. The sums of these respective parameters have been used in predicting the equivalent acid detergent fibre, neutral detergent fibre and acid detergent lignin. Since hemicelluloses, cellulose and lignin are regarded as the component parts of the crude fibre, acid detergent fibre indicates the amount of the plant cell wall that is made up of cellulose and lignin, the neutral detergent fibre tells how much is hemicelluloses and cellulose, while the acid detergent lignin indicates the amount that is pure lignin alone. ADF and ADL represents the most indigestible part of the plant materials. This study shows that the cell walls of the Moringa seeds studied contained very little of completely indigestible plant material (ADF and ADL) and high amounts of the neutral detergent fibre. The high NDF levels among the treatments in this study implied that the crude fibre of Moringa seeds could serves as good sources of short chain fatty acids when fed to herbivorous animals and a good basal ration for monogastric animals. The values obtained in this study showed that as the soaking time increase, the level of the fibre fractions (ADF, ADL and NDF) also increased with the soaking hour. This is in agreement with Makkar and Becker (2007) and Ogbe and Affiku (2012) who reported that the values of fibre fractions in Moringa seeds increased with different processing methods (30 minutes water-soaked, oven-dried, heat treated and autoclaved). They recommended with the results obtained that moringa seeds can be used as feed in poultry nutrition. It is always assumed that the residual fibre in the feaces is directly comparable with that from the original material fed to the animals. This seems to be a doubtful assumption that should be submitted to a careful examination. The changes that takes place during its passage through the digestive tract, the partial utilisation of cellulose, the extensive removal of starch, hemicelluloses and protein completely changes as the

nature of the materials, Ologhobo (1980). Accordingly, Abdelsamie *et al*. (1983) reported that when a crude fibre determination is carried out on the feaces, after for example, a meal of lima-bean, it is hardly likely that the percentage of the residual cellulose and lignin (NDF) that is excreted will be the same as the material originally fed. It is only the acid detergent lignin (ADL) and acid detergent fibre (ADF), less come of its cellulose that will essentially remain undigested.

5.5 Amino Acids Determination of *Moringa oleifera* Seed

The adequacy of a protein for man or farm animals depends on the content of essential amino acids in the protein and the availability of the amino acids. Amino acids have been reported to have a marked effect on the metabolic activities in the cells. Animal kept for several days on a protein deficient but high caloric diet showed a reduced capacity for protein synthesis even though polyribosomes were present in the cells in sufficient amounts. The lack of essential amino acids and the presence of inhibitory component in the cells are also held responsible for the decrease in protein synthesism (Liener 2005). However, imbalance mixtures of amino acids are less effective even at high concentration of protein. Evidence has shown that the inhibitory responses of the mixture represented the summation of effect of the individual amino acids, the most potent of which are methionine and lysine in seeds (Ologhobo *et al*. 2004). This incomplete mixture of amino acids and in-balance of amino acids induced by dietary means can rapidly affect the degree of protein synthesis. For instance, methionine deficiency can be induced in man and rats by feeding raw *Moringa oleifera* seeds. Some of the observed responses in this study may therefore be traceable to a combination of these factors.

The Food and Agricultural Organisation (1994) have set a pattern of the minimum quantities of most of the essential amino acids that a protein should contain to meet dietary requirements. According to this pattern, the processed *Moringa oleifera* seeds studied in this research work were not deficient in the sulphur amino acids, histidine, isolecine, lysine, methionine, phenylalanine, valine and cysteine but these amino acids were at the borderline as reported by FAO (1994). Also, Makkar and Becker (2007), Olugbemi *et al*. (2010) and Ogbe and Affiku (2012), reported earlier that processed *Moringa oleifera* seed is high in sulphur amino acids. This

is contrary to the study of Ologhobo *et al.* (2004) who processed lima bean and cowpea and reported that the amino acids were not in border line as reported by the pattern of FAO (1994). Though the values obtained for leucine, threonine, trypophan, and alannine were slightly deficient from the pattern reported by FAO (1994). All the amino acids values reported by this study were comparable with those reported by Fugile (2004) except for lysine, methionine and histidine, these low values he reported could be due to the fact that he analysed the raw Moringa seeds and the high values obtained in this study could be due to the variety and processing method used.

The high amounts of methionine, lysine and histidine amino acids in this study are noteworthy. The reasons for these accumulation could be that, as suggested by Caceres (1991), Makonnen and Drager (1997), Fughie, (2000) and Verma, (2008) that these amino acids plays the role of a common intermediate in the synthesis of other amino acids, and they often accumulates in seeds and other storage organs where they provides readily available storage form of nitrogen for protein synthesis and a mechanism for the utilisation of ammonia.

Since sulphur containing amino acids methionine and cystine values obtained in this study are within the border line of FAO (1994) pattern, it is hope that this study will have a further study to carry out other processing methods which would have a higher content of these amino acids than has earlier been reported by this researcher and other workers. Though these values could be at the borderline reported by FAO (1994), they are still much higher than the values obtained in soybean and full-fat soyabean according to the reports of Ologhobo *et al*. (2006) who reported that soyabean and full-fat soyabean are limiting mostly in methionine and deficient in lysine.

5.6 Fatty Acid Determinations of *Moringa oleifera* Seed

Results from this study shown that *Moringa oleifera* seed is very rich in total fat. The fatty acids profile of *Moringa oleifera* seeds however, indicate them as very good sources of oleic, palmitic, behenic, stearic and arachidic acids. Generally, linolenic acid, myristic acid, palmitic acid, eicosenoic acid and lignoceric acid have been found to only occur in small quantities in both the raw (T1) and the water-soaked *Moringa* seeds (T2, T3 and T4). Both the raw MOS and the

water-soaked MOS are good sources of oleic, Behenic, palmitic and steric fatty acids, four in palmitolic, eicosenoic, lignoceric and arachidic fatty acids, but poorer in linolenic and myristic fatty acids. Although the present results are too limited to permit any generalisation from them, it is tempting to speculate that there may be a closer genetic relationship between the water and *Moringa* seeds.

When the results of this study are compared from the point of view of the un-saturation, the raw (T1) MOS can be said to better sources of all the fatty acids analysed, followed by the 3 hours water-soaked MOS (T4). The physiological importance of these unsaturated fatty acids lies in the ability of the body to synthesised them at least adequately. This study is in agreement of the report of Caceres, (1991) who reported that *Moringa oleifera* seeds are rich in unsaturated fatty acid and has 72% oleic acid which is similar to that for olive oil. Guo *et al*. (2003) and Makkar and Becker (2007) also claimed that both raw and processed *Moringa oleifera* seeds are good profile of fatty acids contents. Ologhobo (1980) reported lower values for oleic acid, palmitic acid linoleic acid on cowpea, soyabean and full-fat soyabean, and he claimed that these acids are very poor in the total fat of each of the nutrient. The lower value he obtained for full-fat soyabean could be as a result of variety and the processing methods he used. Makkar and Becker (2007) reported 56% total fat of *Moringa oleifera* seeds while 48% was recorded for full-fat soya by FAO (1994). The fatty acids profile in this study were better than the reports of Fugile (2004) and Verma (2008).

The polyunsaturated fatty acids are readily oxidized their excessive intake which will increase the requirement for vitamin E which appears to serve primarily as an antioxidant in the body. Several experiments have shown that the levels of vitamin E will normally dystrophy and ene-ephalomalacia, which will prove inadequate as the intake of the essential fatty acids will be increased.

Adams *et a*l., (2009), reported that when calves were fed certain vegetable oils as supplement to skin milk, poor results were obtained compared with those where butter fat was used. Statement can be made from the results of this study that processed *Moringa oleifera* seeds can be fed to

man and animals even at larger quantities because apart from providing sizable amount of dietary fat, they will also increase the requirement for vitamin E. though there are no documents to support this assertation, but there will be further research about it.

5.7 Performance Characteristics of Albino Rats Fed *Moringa* Seed Meal

The values of the total fed intake showed significant differences (P>0.05) when fed MOSM to albino rats. The total feed intake on the control diet (T_1) as the sole protein source was significantly better than those on the tested diets. Casein is a pure protein with well balanced amino acid profile. The higher feed intake for rats on casein diet indicated that the diet was more palatable to the rats. The low feed intake in the tested diets could be due to poor palatability. The more palatable a diet is, the more it is acceptable and consumed and vice versa. Feed intake is associated with nitrogen source, palatability, flavor and amino acid (Chikwendu and Obizoba, 2003). The low intake in the dietary treatments could be attributed to the low solubilisation of some anti-nutritionals factors that are still present in both the raw and water-soaked *Moringa oleifera* seed meal.

The average weight gained by rats fed on casein diet were comparable to the weight gained by rats fed on diets 2, 3. These weights were significantly (P>0.05) better than the weights of rats fed on non-protein diet. The weight gain obtained in rats on diet 3 (water-soaked MOSM) indicated that 3 hours processing method employed was effective in eliminating the anti-nutritional factors to a tolerable level, thereby improving the protein availability to favourable level hence, relatively remarkable food intake and body weight changes. This study is in agreement with the report of Ruada *et al.* (2003) who observed low weight gain in rats when fed raw and treatment jack bean, they attributed the low weight gain to some anti-nutritional factors that were still present in the tested diets. Also Ologhobo *et al*. (2006) observed low weight gain in rats fed processed lima bean and cowpea, they also attributed poor weight gain to the presence of toxic factors particularly, haemagglutinins. Amusa, (2011) observed higher weight gain when he soaked dehulled jack beans in Ogi solution, and reported significant influence on nutrient and anti-nutrient utilisation of Jack bean, but he reported low weight gain in rats fed water-soaked

and jack bean soaked in "Ogi" liquour. The lowest weight gained by rats fed diet 4 (protein free diet) is in agreement with the result of Obizoba (2006), who observed a similar low weight gain in rats fed 80% sorghum and 20% grain legume. The lower weight gain may be probably due to low protein intake to synthesize the body tissue. However, the results of the weight gain of the experimental animals were partly influenced by feed intake and by the essential amino acid pattern of the dietary protein fed to the animals. This result showed a linear relationship between changes in weight and quality of feed consumed and protein intake. The same trend of result was observed by Said and Hegsted (2009).

The feed conversion ratio (FCR) showed casein meal diet (T_1) to be the best diet in terms of feed, since it gave the lowest FCR value. This value was comparable in rats fed diet 2, and 3 and were significantly better ($P<0.05$) than rats fed diet 4 (protein free diet). The comparable values of FCR in rats fed diets 2 and with casein may be probably due to the fact that each of these diets consisted of protein which complemented each other in terms of essential amino acid availability.

5.8 Protein Quality *Moringa* Seed Meal Fed To Albino Rats

The values obtained for these diets in protein efficiency ratio were significantly higher ($P>0.05$) in rats fed the casein diet (T_1) than other diets. This may also be due to the presence of complete essential amino acids in the diet. The highest numerical value of PER after casein diet was observed in the rats fed 3 hours water-soaked MOSM (T_3), which consisted of processed plant sources of protein which perfectly complemented each other in terms of amino acids balance. This is in agreement with Ketiku and Smith (2004) who observed an increased in the PER of cereal when fish meal was added to the diets. The high value of PER in diet 3 (3 hours water-soaked MOSM) which favourably compared with that of casein diet (T1), also agrees with Akomolafe (2008) who recorded higher values of PER in a feed mixture of cowpea-Ogi and maize-groundnut. Olusanya (2008) also recorded a comparable PER for maize-beans, amaranthus mixture and casein in diet. However, high value of PER obtained for diets 1 and 3 over diets 2 and 4 has been confirmed by Akinrele and Edwards (2007) and Ketiku and Smith

(2004) who showed that maize-groundnut mixture have higher PER value compared to soyabean-ogi. However, statistically higher value of PER for diet (3) which consisted of 3 hours water-soaked MOSM over diets 2 and 4 is also in agreement with Ifon (2008) who recorded an improved PER when millet porridge was fortified with soyabean.

The higher values of NPR and PRE were observed in rats fed casein diet (T1) followed by rats fed water-soaked MOSM (T3). The high net protein retention (NPR) value of diet 3 compared to diets 2 and 4 is probably due to the processing method used in the *M. oleifera* seeds. It has been recorded by various workers, Smith (2008) and Akomolafe (2008) that fortification of cereal with protein from animal or plant sources particularly soyabean and fish meal usually improved its nutritional quality. Ene-Obong and Obizoba (2005) confirmed crayfish protein as a better supplement to legume/cereal than leguminous oil seed, which is far contrary to the results in this study. Water-soaked MOSM obtained better protein utilisation than the raw and very next to the rats on casein diet.

The biological value (BV) and net protein utilisation (NPU) of this study revealed that the nutritional values of the raw and water-soaked MOSM fell below the digestibility value than the casein diet, this result is similar to the report of Ologhobo (2006) and Olusanya (2008), who observed lower values in BV and NPU when rats fed with processed lima bean and cowpea, respectively. According to Melnick and Cargill (1949), this observation might be due to delayed release of a particular amino acid such that mutual supplementation between essential amino acid was impaired. With respect to the NPU values, rats fed on diet 3 (water-soaked MOSM) tended to have a comparable value with that of casein diet (T1), indicating that the feeds consumed were will utilised by the experimental animals. This was further confirmed by the NPU values being higher than the 60% value recommended by NRC (1984) for weaning foods and being significantly ($P<0.05$) different from diets 2 and 4 which falls below the recommended value by NRC (1984).

The similarity in biological value for the rats fed casein diet (T1) and diet containing water-soaked MOSM (T3) seems to suggest that these diets provided a good combination of essential

amino acids which were available for body tissue synthesis. The poor biological value of the protein free diet (T4) coupled with the low value for NPU were clear indications of the lack of protein source in the diet compared to the other diets.

5.9 Haematological Indices of Albino Rats Fed *Moringa* Seed Meal

The PCV values obtained in this study were significantly ($P<0.05$) different among treatments. Packed cell volume is an index of toxicity, any reduction in its concentration in the blood would usually suggest presence of toxic factors which has adverse effect on blood formation (Oyawole and Ogunkunle, 1998). Raw MOSM has been reported to contain some anti-nutritional factors, it is possible that traces of these anti-nutritional factors still present in the water-soaked MOSM and may be perhaps have been responsible for the value obtained in this study. It could also be that the 3 hours water-soaked method was not able to completely remove the anti-nutritional in the diets. The higher the PCV value, the more capable the processing method. This implies that 3 hours water-soaked *Moringa* seeds compared with the control as against the un-processed ones.

Haemoglobin values ranges from 13.33-10.13 were significantly ($P<0.05$) different among treatment groups. The low level of haemoglobin of the tested diets compare with the casein diet implies that there were probably traces of anti-nutritional factors in the tested diets. This study agrees with the report of Akinmutimi, (2004) that processing methods reduces but do not completely eliminate all traces of anti-nutritional factors in feed stuffs. Diet containing protein-free diet usually influence poor transportation of oxygen from the respiratory organs to the peripheral tissues (Roberts *et al*., 2004).

The red blood cell values of rats on casein diet (T1), raw MOSM (T2) and water-soaked MOSM (T3) were significantly ($P<0.05$) different from the rats fed protein free diet (T4). Rats on casein diet obtained the highest value on RBC, the observed decreased in the RBC values of rats on protein free diet (T4) T2 could be attributed to the destruction of RBC by the residual anti-nutritional factors and toxicants in the feeds. This is in agreement with the study of Oyawole and Ogunkunle (1998) who reported that biochemical components of blood are sensitive to element

of toxicity in feeds. However, haematological components are also valuable in monitoring feed toxicity especially with feed constituents that affects the formation of blood, Ahmed (2011).

The significant ($P<0.05$) difference in white blood cells suggested that there were microbial infections or the presence of foreign body or antigen in the circulatory system. The high levels of lumphocytes, neutrophills and monocytes in rats fed raw MOSM (T2) is an indication of chronic inflammation in nutrient present in the raw seeds. However, this study is in agreement with Akangbe (2011) who reported that inclusion of *Moringa* leaf meal at 2% in the diet of laying chickens increased PCV and RBC. The report of Kurma and Mishra (2003) did not agree with this results when they included *Moringa pterygosperma* at low inclusion levels of 0.2%, 0.4% and 0.6% and obtained low values in white blood cell differential counts.

Significant ($P<0.05$) differences existed in the values of mean corpuscualar volume (MCV) and Mean corpuscular haemoglobin (MCH). The values obtained for MCV and MCH followed a similar trend as there was significant difference between rats on protein free diet (T4) and other treatments. This may be due to poor values of packed-cell volume, red blood cell and haemoglobin, since the values of MCV and MCH obtained in T4 are functions of the values suggest nutritional inadequacy of the diet (Abu *et al*., 1998, Onwukwe, 2000).

5.10 Serum Biochemical Variables of Albino Rats Fed *Moringa* Seed Meal

The values obtained on serum biochemistry varied significantly ($P<0.05$) between treatments. It was observed that rats on casein diet (T1) had the highest total protein value than other treatments while rats on protein free diet (T4) obtained the lowest total protein values while rats on MOSM had the middle values. This shows that the health status of the rats on both the casein and *Moringa* diets maintained favourable health status.

The albumin and globulin values of rats fed the casein diets (T1) raw MOSM (T2) and water-soaked MOSM (T3) obtained the highest values which signified that these treatments have the ability to fight against diseases (Roberts *et al*., 2003).

The cholesterol values were significantly ($P<0.05$) different in the tested diets (raw and water-soaked MOSM) than the casein and the protein free diets. Rats fed water-soaked MOSM (T3) obtained the lowest cholesterol value followed by rats fed raw MOSM (T2), this is an indication that the seed meal has effect in the serum lipids of the rats which shows that *Moringa* seed meal reduced the cholesterol contents in rats as earlier reported by Guo *et al.* (2003), Ogbe Affiku (2012) and Annogu *et al*. (2013). This study is also in agreement with the report of Nwanjo (2007) who fed phyllanthus anarus extract to diabetic rats and concluded that there were reductions in the cholesterol values on the rats. The high cholesterol value in rats on casein diet (T1) shows that the diet consumed was high in lipid which gave higher increased in the cholesterol contents, same applied to rats on protein free diet (T4).

The results of this study shows that the reduction in values for AST and ALT on rats fed *Moringa* seed based diets (T2 and T3) could be inferred that *Moringa oleifera* seed meal have some protective effect on the liver. Liver protective medicinal plants have shown to contain a variety of phytochemical constituents (Gupta and Misra, 2008). These phytochemicals are also present in *Moringa oleifera* seeds which may be responsible for the results obtained in this study. It can be inferred that the seed meal of *Moringa oleifera* have appreciable ability to prevent damage to the liver (Adedapo *et al*., 2009). The liver enzymes are normally present at low levels in the blood, so if the liver cells are damaged, it would be expected that some of the enzymes could leak into the blood and increase in levels and this can be related to the high values obtained in rats on casein (T1) and protein-free diets (T4), which indicates possible hepatic damage since these enzymes are produced by the liver and also in other parts of the body as in the case of AST (Crook, 2006; Adedapo *et al*., 2009).

5.11 Histology of Albino Rats fed *Moringa* Seed Meal

Histology evaluation of the kidneys fed water-soaked MOSM (T3) showed normal kidney architectures, there were no distortions or inflammatory cells observed, this could be due to the effect of the processed *Moringa* seeds. This study is in line with the studies of Ewuola (2009) and Aja *et al*, (2014) who established that there were no inflammatory cells observed in treated

Moringa seed and lima bean respectively. The mild kidneys observed in other treatments could be due to the toxic effects in the diets of the animals.

The histology of the liver of rats in this study showed significant ($P<0.05$) different among treatments. Rats fed water-soaked MOSM (T3) and rats on protein-free diet (T4) were observed to have mild necrosis in their liver cells while rats fed the casein diet (T1) and rats fed raw MOSM (T2) presented moderate necrosis in their liver cells. The mild necrosis showed that the diets were tolerable by the rats as there were no much toxic effects observed, which was comparable to the rats with moderate necrosis in their liver cells. The moderate necrosis could be an indicator of liver damage resulting from accumulation of fat deposit and toxicity in the raw MOSM. This result is not in agreement with Nikkon *et al*. (2009) and Desta *et al*. (2012) who established that raw *Moringa oleifera* seed meal did not have adverse effects on the liver cells when fed to rats. Mekonnen *et al*. (2005) and Adedapo *et al*. (2009) reported that treated *Moringa oleifera* seed meal when fed to rats did not show any morphological changes in their cells. This result is also substantiated by the results of biochemical assays of the blood of rats administered *Moringa oleifera* seed meal such as AST and ALT which are the main indicators of liver damage.

5.12 Proximate Compositions of Feed Samples

The proximate composition of the test diets for starters and finishers showed that complete replacement of full-fat soyabean with *Moringa oleifera* seed meal (T5) resulted in a diet that had highest crude protein, crude fibre and ether extracts, while the control diet (T1) had the least values than other treatments. This study is in agreement with Kakengi *et al*. (2007) who replaced *Moringa* seed meal with sunflower seed meal in the diets of laying hens, Akintunde *et al*. (2013) in local chickens and Annogu *et al*. (2013) in broiler chickens, at different inclusion levels. These authors reported that there were increased in the values of crude protein, crude fibre and ether extract when *Moringa* seed meal was included in the diets of monogastics animals.

The values obtained in this study is in line with Annogu *et al*. (2013) who claimed that dietary fibre and protein digestibility increased in broiler chickens fed MOSM. However Abdelsamie *et al*. (1983) had a contrary view, that high fibre decreased animal feed intake and nutrient digestibility through changes in the gut, while Varel *et al.* (1988) concluded that there was inverse relationship between dietary fibre and digestibility coefficient of nutrients.

5.13 Performance Characteristics of Broiler Chickens Fed *Moringa oleifera* Seed

The higher body weight gain and feed intake of the control diets may be due to low fibre compared with other diets, Malessesal *et al*. (2011) had earlier reported that low fibre intake significantly effect weight gain and feed intake in the diets of broiler chickens. The feed conversion ratios, birds on control diet were able to utilised the feed better probably because the fibre level of full-fat soyabean was lower compared to other diets. *Moringa oleifera* seed is higher in fibre than full-fat soyabean.

5.14 Nutrient Digestibility of Broiler Chickens Fed *Moringa oleifera* Seed

Among birds on the test diets, birds on treatment 1 (100% MOSM) had a better mean digestibility value than those on other diets. The values obtained in this study is in line with Annogu *et al*. (2013) who claimed that dietary fibre and protein increased the diet of broiler chickens fed MOSM. Abdelsamie *et al*. (1983) had a contrary view with this study, they reported that high fibre decreased animal feed intake and nutrient digestibility through changes in the gut, while Varel *et al*. (1988) concluded that there was an inverse relationship between dietary fibre and digestibility coefficient of nutrients.

5.15 Haematological Indices of Broiler Chickens Fed *Moringa oleifera* Seed

The increased in the values of PCV, hemoglobin and RBC an indication of the influence of MOSM in increasing the size of the erythrocytes which aid and improved the erythrocyte

morphologically because the birds were not anaemic and increase in the erythrocytes leads to increase level of hemoglobin which is erythrocytes compare with Mitruka and Rawnsley (1977). The values obtained from the WBC differential counts (lymphocytes, Heterophils, Monocytes, Eosinophils and Basophils) were still within the normal values as reported by Mitruka and Rawnsley (1977) which is an indication that the seed meal had no detrimental effects on the animals. These results is also in line with the study of Esonu *et al*. (2001) who fed *Mucuna* seed meal to weanling pigs and concluded that their findings were in agreement with Mitruka and Rawnsley (1977) on haematological indices. They stated that haematological constituents reflect the physiological responsiveness of the animal to its internal and external environments which includes feeds and feeding. This study is also in line with the reports of Onu Aniebo (2011), who reported that *Moringa* seed meal increased the values of PCV, RBC and Hemoglobin counts when fed to broiler chickens at 2.5%, 5.0% and 7.5% inclusion levels. They concluded that the values obtained in all the treatments groups indicated nutritional adequacy of all the diets and presence of toxic factor since values did not indicate mal-or under nutrition. PCV is an index of toxicity reduction in the blood (Church *et al*., 1991).

5.16 Serum Biochemical Variables of Broiler Chickens Fed *Moringa oleifera* Seed

Based on these results obtained, it shows that *Moringa oleifera* seed meal have some degree of hepatoprotective ability. It can be inferred that MOSM have some protective effects on the liver as shown by the reduction in the levels of the liver enzymes. Gupta and Misra (2006) reported that liver protective herbal drugs have been shown to contain a variety of chemical constituents such as phenols, alkaloids, tannins, glycosides, carotinoids, lipids, essentials oils etc. Some of these phytochemicals are still present in water-soaked *Moringa* seeds which may be responsible for these effects in this study. It can be inferred that *Moringa* seed meal have appreciable ability

to prevent damage to the liver (Adedapo *et al*. 2009), (Makkar and Becker, 2009). The liver enzymes are normally present at low levels in the blood, so if the liver cells are damaged, it would be expected that some of the enzymes would leak into the blood and increase in levels. These increase in AST, ALT and ALP in the control diet (T1) indicate a positive hepatic damage since these enzymes are produced by the liver and also in other parts of the body (Crook, 2006; Ranjan *et al*., 2009). Values obtained in uric acid and creatinine in the control diet (T1) were significantly higher ($p<0.05$) than other treatments, which means that *Moringa oleifera* seed meal in this study influenced the uric acid and creatinine in broiler chickens. This is not in agreement with Han *et al*. (1998) who reported that full-fat soyabean had no harmful effects on performance and blood profile of broiler chickens. The result obtained for dietary treatments, the value obtained in the control diet did not fall within the range of 162.00-192.00mg/dl reported for chickens by Mitruka and Rawnsley (1977), while values obtained from the dietary treatments are within the normal range. The reduced values observed in the dietary treatments could be as a result of high fibre content in *Moringa* seed meal, this is in agreement with Ogunwole, (2004) who reported that high fibre diet reduced serum glucose concentration. This study also agreed with the report of IFST (2001) who observed that some fibre improved glucose metabolism and insulin response. Diets high in fibre may improve glycometabolic balance in diabetic patients.

5.17 Lipid Profile of Broiler Chickens Fed *Moringa oleifera* Seed

This result is in agreement with the reports of Olugbemi *et al*. (2010), Abiodun *et al*. (2012), Annogu *et al*. (2013) and Sola-Ojo *et al*. (2013) that *Moringa oleifera* leaf and seeds possesses hypoglycemic, hypolipidemic and hypocholesmic properties. The total cholesterol value obtained in the control diet (T1) (206.33(mg/dl) did not fall within the normal range of 59.00-148.00mg/dl as reported by Mitruka and Rawnsley (1977) while the values of the dietary

treatments fall within the normal range as T5 (100% MOSM) obtained the cholesterol value of 112.67(mg/dl) while treatments 2, 3 and 4 had the following cholesterol values of 156.67 (mg/dl), 133.52 (mg/dl) and 129.50 (mg/dl) respectively. The high level of lipid metabolism in the control diet showed that full-fat soyabean is high in ether extract, thereby causing high energy level which resulted in high lipid or fat in the blood cholesterol. While the reduction in cholesterol in the dietary treatments could be as a result of increase in fibre levels in the diet which IFST (2001) hypothesized to be due to the production of fermentation of soluble fibre in the gut which inhibit synthesis of cholesterol by the liver, thus reducing concentration of blood cholesterol. Soluble fibre of the diet may also bind cholesterol in the intestine, preventing its re-absorption into the body therefore reducing the blood cholesterol as observed in birds fed 100% *Moringa oleifera* seed meal.

5.18 Immunoglobulin Assay of Broiler Chickens Fed *Moringa oleifera* Seed

This result is not in agreement with study of Jahanian (2009) who carried out a study on effects of ammonia on serum 1gG, 1gA, 1gM, in broiler starters and reported that serum immunoglobulins concentration declined with the increase of ammonia concentration. In addition, immunoglobulin secretion is a complex metabolic process playing an important role in the process of the immune system to fend off antigen, they concluded that the data shows chronic ammonia stress which affect immunoglobulin stability and decreased 1g levels in high ammonia conditions would result in decreased responses to infections. A study reported by Jayavardhanan *et al*. (1994) is in line with this study. They fed broiler chickens using *Moringa* seed extracts to modulate defense system, and they concluded that there were increase in the immunoglobin 1gs levels and the birds were stress free. This study is also in support of the report of Konashi *et al*.,(2000) who reported that diet high in protein synthesis and muscle deposition has also been

demonstrated to be involved in the synthesis of cytokines, proliferation of lymphocytes and thus in the optimum functioning of immune system in response to infection. Inadequate supply of amino acid will reduce antibody response and cell-mediated immunity in chicken Chen *et al*, (2003).

5.19 Histological Assay of Broiler Chickens Fed *Moringa oleifera* Seed

This result is in line with Adedapo *et al.* (2009) who concluded that *Moringa oleifera* seed extracts have appreciable ability to prevent damage to the heart.

In the kidneys, there were congestion and haemorrhages similar among the birds in all treatments. There were multiple foci of tubular degeneration and necrosis with marked congestion of interstitial blood vessels which resulted to widespread of necrosis of renal tubules. This shows that the kidney were affected negatively which was more severe in T1 (control), T2 (25% MOSM) and T4 (75% MOSM) while T3 (50% MOSM) and T5 (100% MOSM) obtained mild congestion of renal tubules. This result could be that *Moringa oleifera* seeds soaked in water for 3 hours still possess some presence of phytochemicals which resulted in renal break down. This study is in line with the reports of Makkar and Becker (2009), Ogbe *et al*. (2012) who concluded that raw *Moringa oleifera* seed fed raw to poultry will damage the organs especially the kidneys and levels.

The liver results showed that T1 (control), T2 (25% MOSM) and T3 (50% MOSM) obtained mild and moderate in their liver cells. This showed that the hepatic plates were slightly separated from each other resulting to few foci of mild and moderate hepatocellular necrosis with aggregates of heterophils at the portal tracts, while T4 (75% MOSM) and T5 (100% MOSM) maintained normal functioning in their hepatic cords. Liver enlargement due to toxic effects in seeds has been reported in other animals including fish and rats (Ologhobo *et al*., 2006, Kawo 2009, Ahmed 2011, Bundit *et al*. 2014). Significantly, higher score of liver lesion occurred due to high level of full-fat soyabean (100%FFSb, 75%FFSb and 50%FFSb) in the diets. However, mild histopathological changes in the liver were observed in birds fed 75% MOSM and 100%

MOSM, this study is in agreement with Bundit *et al*. (2014), who reported mild histopathological changes in the liver of Bocourti's catfish when fed with 50% and 75% of MOSM as replacement for SBM. Also Mekonnen *et al.* (2005) reported that *Moringa oleifera* seed extracts did not affect the liver cells when fed to broiler chickens. The results of this study suggests that *Moringa oleifera* seed meal can be included in broilers diets at a proportion of not over 75% replacement protein in full-fat soyabean without inducing morphological changes in the liver.

5.20 Carcass Characteristics of Broiler Chickens Fed *Moringa oleifera* Seed

This study is in agreement in the finding of Affiku and Rasheed (1996), Esonu *et al*. (2006) and Melesse *et al*. (2011). From this study it was observed that birds on control diet obtained the highest values than other treatments which was significantly different ($p<0.05$) than others. These differences lies in the values of concentration of MOSM. These authors, also used high inclusion levels suggesting that *Moringa* seed powder above 5% in a broiler diets will result in dress weight % reduction as a result of depletion of the energy in the muscle tissues (Esonu *et al.* 2006).

5.21 Primal Cuts of Broiler Chickens Fed *Moringa oleifera* Seed

The relationship among treatment groups live weight and cut parts changed significantly as the increase in the concentration of *Moringa* seed meal. The relative weight of chest, wings, thigh, drumstick and back of birds fed the control diets were significantly higher ($p<0.05$) while birds fed 100%MOSM (T5) had the least values, this could be as a result of the final weights and carcass weights, this study is in agreement with the reports of Annogu *et al*. (2013) who observed that *Moringa oleifera* seed powder depressed performance and cut parts of broiler chickens fed up to 15% inclusion of *Moringa* seed powder, but Oyedele *et al*. (2013) observed better performance in weight gain and cut parts when fed MOSM to local chickens at 5%

inclusion level. The decreased in the cut parts values of the dietary treatments could be as a result of the high level of inclusion of MOSM in this study.

5.22 Organ Characteristics of Broiler Chickens Fed *Moringa oleifera* Seed

The heart showed significant difference as the birds in control diet had the least value of 0.20% than other treatments with values ranged from 0.39 -0.56%, where T5 (100%MOSM) obtained the highest value, this could be as a result of expansion in the heart caused by toxic substances in the seed meal. But for abdominal fat pad, birds on 100% FFSB (T1) obtained the highest value which was quite different from the moringa treatments with the value of 4.83 compared to 2.97% in birds fed 100% MOSM. The liver, lung and kidney weight of birds fed 25% MOSM (T2)–100%MOSM (T5) were bigger than those fed the control diet. This might be as a result of the increase in the activity of those organs having a prolonged consumption of NSP which was reported by Esonu *et al*. (2006), Nguyen *et al*. (2012) that increase in the organ weight relative to the body weight of the animal will reduce feed utilisation which will lead to enlargement of digestive organs as noticed in this study. Increase in internal organs like the liver, kidney and lungs may lead to decreased relative carcass weight. The liver is considered the primary target organ for plant toxicity (Nguyen *et al*., 2012). In this study, the increase in weight of the liver and kidney could be attributed to gross hepatic changes produced by some of the phytochemicals that were still present in the *Moringa oleifera* seed meal even after water-soaked for three hours, The high spleen weights of birds on the dietary treatments were however contrary to the report of Wang Xin-hau *et al*. (2001) who stated that low spleen weight normalised the spleen weight of patients fed *phyllanthus anarus*.

The weight of the intestine increased with increased in inclusion level of MOSM, where birds on the dietary treatments obtained the highest values. This study is in agreement with Abdelsamie *et al*. (1983) who reported increased in weight of the gastrointestinal tract in broiler chickens, when fed with diets high in fibre.

The highest values on the weights of gizzards and proventiculus on birds in T5 (100%MOSM) showed that there were increased in the muscular activity which could be as a result of increased in crude fibre. This result is in line with the report of Summer and Leeson (1986), Karuna *et al*. (2011) who observed increased in gizzard weight of broiler chickens when fed with diets high in fibre. This increased value which also led to the increased in the weight of liver and kidney.

5.23 Proximate Compositions of Meat Samples of Broiler Chickens Fed *Moringa oleifera* Seed

This study is in agreement with Makkar and Becker (2009) who reported that *Moringa oleifera* seeds soaked in water for 20-30 minutes will increase the crude protein, ash contents and reduced the ether extract of broilers meat. Also Mupangwa *et al*. (2010) treated *Moringa* seeds with heat and included in the diets of Nile Tilapia fish at 0,5 and 10.0% levels, they concluded that 10% inclusion level increased the crude protein and Ash contents of Tilapia fish and reduced the fat content and dry matter levels.

5.24 Sensory Evaluation of Broiler Chickens Fed *Moringa oleifera* Seed

These increased in aroma values will be as a result of the Aromatic substances in the seed meal as reported by Makkar and Becker (2009) who observed some attractive aromatic substances in water-soaked *Moringa oleifera* seed meal.

Meat on T1 (control) obtained the highest values on flavor, tenderness, thickness and over all acceptability than other treatments, while treatment 1 (100%MOSM) obtained the least values. These results shows that organoleptic test approves T1 (control) and T2 (25%MOSM) acceptable for human consumptions

CHAPTER SIX

SUMMARY AND CONCLUSION

Three experiments were conducted to determine the utilisation of *Moringa oleifera* seed as alternative to full-fat soyabean in the diets of broiler chickens. The first study involved the determination of chemical characterisation of raw and water-soaked *Moringa oleifera* seed after subjecting it to different hours of water-soaking and chemical treatments. The second study evaluated the dietary protein quality of raw and water-soaked *Moringa oleifera* seed using albino rats in which the three hours water-soaked *Moringa oleifera* seed was used to compare with the raw seed. I n experiment three, *Moringa oleifera* seed was incorporated into the diets of broiler chickens at 0, 25, 50, 75, and 100% inclusion levels.

In experiment one, chemical characterisation of raw and water-soaked *Moringa oleifera* seeds were determined. The results indicated that:

1. The different hours of soaking to which moringa seed was subjected to have effect on both the nutrient and chemical compositions of the moringa seed.
2. There was an increase of 36.08% in the crude protein content of moringa seed when it was soaked for three hours compared to the unsoaked (35.40%).
3. The phytochemical contents reduced with an increase in the hours of soaking. Three hours water-soaked moringa seed had the lowest phytochemical values when compared to the raw and other hours of soaking.
4. The amino acids and fatty acids profile values were also increased with the soaking hours but the raw obtained the highest mean values.

In study two, 10% crude protein of the raw and three hours water-soaked moringa was used to evaluate the protein quality using albino rats, the results obtained indicated:

1. There were significant differences (P<0.05) on the performance as three hours water-soaked moringa seed obtained the highest feed intake (41.75g) and weight gain (9.20g) compared to the raw.
2. Rats fed on three hours water-soaked moringa seed had better protein utilisation than the rats on the raw moringa seed diet.
3. There were significant differences (P<0.05) on the biological values as rats on the three hours water-soaked were able to effectively utilised the protein in the diets.

In study three, *Moringa oleifera* seed meal was incorporated into the diets of broiler chickens at, 0, 25, 50, 75 and 100% inclusions and the results indicated:

1. There were reduction in feed intake and weight gain in birds on the moringa treatments compared to birds fed 100% full-fat soyabean.
2. Birds fed 100% moringa seed had better mean digestibility values than other treatments.
3. Birds on 100% full-fat soyabean diets had the highest carcass weights, while birds on 100% moringa seeds had the lowest abdominal fat pad. Moringa seed had lipogenic effect.
4. The birds fed with the dietary treatments had a better result on organ toxicity which shows that moringa seeds have some degree of hepato-protective ability
5. *Moringa oleifera* seed lowered the cholesterol level in birds fed the dietary treatments and also boosted their immunoglobulin system

In conclusion *Moringa oleifera* seed soaked in water for three hours had significant influence of nutrients and anti nutrients compositions because *M.oleifera* seed contained appreciable amounts of protein, minerals, and amino acids, which are nutritional poultry requirements. Thus, *moringa* seed can lower the production cost in poultry diets and add value to a plant origin. From the rat study, its also showed that *moringa* seed soaked in water for three hours were able to meet up with the protein quality.

It is therefore concluded that *moringa* seed even at 50% inclusion in broilers diet had no adverse effects on organs toxicity and blood chemistry.

CHAPTER SEVEN

RECOMMENDATIONS

Moringa oleifera seeds should not be used raw in formulating feeds for poultry because of the inherent anti-nutritional factors. However soaking in water for up to three hours was not sufficient fot the removal of a greater part of the ANFs. Inclusion of 3-hr water-soaked *Moringa oleifera* seed at 50.0% can replace 50.0% full-fat soyabean in broiler diets without reduction in the performance of broiler birds.

With the possibility of some anti-nutritional factors that may still be present in the dietary inclusions, other processing methods can also be used in future studies to reduce the anti-nutritional factors, thereby enhance poultry production.

To drive the Nigeria livestock industry the use of processed moringa seeds may also play a significant role in the diets of other livestock species.. This drive has often been curtailed by scarcity and or exorbitant prices of certain feed ingredients, particularly the animal protein concentrates. With the findings from this study, it ought to be possible for livestock farmers to choose and utilise these non conventional protein feed ingredients in the absence of the conventional ones.

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