GROWTH PERFORMANCE AND CALCIUM RETENTION OF BROILER CHICKENS FED DIETARY OILS

BY

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ABSTRACT

Dietary Oils (DO) are used in poultry feeding without considering their effects on nutrient digestion, absorption and utilisation. Oils rich in Saturated Fatty Acids (SFA) may interfere with uptake of minerals especially calcium in chickens' gut, resulting in the formation of indigestible soaps which reduce absorption of nutrients. Characterisation of DO is imperative to ascertain the likely effects on nutrients uptake. However, there is dearth of information on the effects of different DO on performance, serum biochemical indices and calcium retention in Broiler Chickens (BC). Therefore, effects of DO and varying Dietary Calcium (DC) levels on performance and calcium retention in BC were investigated.

Five DO: Shea butter-Sb, Soybean Oil-SO, Groundnut Oil-GO, Coconut Oil-CO and Palm Kernel Oil-PKO were analysed for Arachidonic, Oleic, palmitic, total carotene and α-tocopherol concentration (μ/mL) using standard procedures. Nine diets with three DO based on degrees of unsaturation containing 1%, 2%, 3% each of Sb (T_1, T_2 and T_3), PKO (T_4, T_5 and T_6) and CO (T_7, T_8 and T_9) were formulated. Arbor Acre BC (n=360) were fed the diets for six weeks. Weight Gain-WG (g/bird) and Feed Conversion Ratio (FCR) were calculated. At day 21, blood (3 mL) was sampled from two BC/replicate for serum biochemical assay using standard procedures. Another 540 BC were allotted to nine dietary treatments comprising 2% PKO and DC at 0.75 (T_A), 1.00 (T_B)and 1.25% (T_C); 2% Sb and DC at 0.75 (T_D), 1.00 (T_E), 1.25% (T_F); 2% CO and 0.75 (T_G), 1.00 (T_H), and 1.25% (T_I), for six weeks. Two BC/replicate were housed in metabolic cages from day 35 to 42 for the determination of calcium retention and left tibia ash using standard procedures. The experiments were 3x3 factorial arrangements in a completely randomised design. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Arachidonic acid concentration in GO (0.07±0.01) and Sb (0.08±0.02) were significantly lower than SO (2.33±0.05), PKO (2.10±0.03) and CO (2.25±0.15). Oleic acid in CO (26.95±2.57) and Sb (26.93±1.78) were significantly lower than 42.15±1.35 (GO), 38.65±4.17 (PKO) and 37.67±2.30 (SO). Palmitic acid ranged from 3.45±0.57 (Sb) to 8.83±0.89 (SO). Total carotene in Sb (8550.26±280.00) and CO (8345.06±203.00) were significantly higher than 3396.54±133.86 (PKO), 1641.48±72.50 (SO) and 4372.67±165.17 (GO). The α-tocopherol ranged from

25.59±0.35 (PKO) to 53.37±2.18 (Sb). The WG of BC fed T_1 (924.38±57.25) T_2 (881.38±40.77), T_5 (936.50±60.13), T_6 (882.25±44.65), T_8 (959.50±58.02) and T_9 (904.38±50.01) were significantly higher than 788.13±34.20 (T_3), 756.00±20.25 (T_4)and 856.75±32.05 (T_7). The FCR ranged from 2.37±0.05 (T_1) to 2.55±0.04 (T_5). Superoxide dismutase (µL) was highest in BC fed T_9 (174.00±0.14) and least in T_1 (147.75±19.20). Serum calcium (mg/dL) ranged from 12.88±0.04 (T_1) to 14.40±0.46 (T_7). The BC on T_I (92.62±4.02) had highest calcium retention and in T_E (49.55±1.85) was least. Tibia ash (%) was highest in BC fed T_H (86.38±5.75) and least in T_B (77.86±3.25).

Inclusion of shea butter at 1%, palm kernel and coconut oils at 2% enhanced weight gain. Combined dietary inclusion of 1.25% calcium and 2% coconut oil improved calcium retention in broiler chicken.

Keywords: Dietary oils, Polyunsaturated fatty acids, Calcium digestibility, Tibia ash, Total carotene.

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Abokede Titilope V.

166009

DEDICATION

I dedicate this Thesis to the Lord Almighty and my loving son, Daniel Oluwadamilare Ishola.

CERTIFICATION

I hereby certify that this work was executed by Titilope Victoria ABOKEDE in the Agricutural Biochemistry and Nutrition Unit, Department of Animal Science, Faculty of Agriculture, University of Ibadan, Ibadan

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CHAPTER ONE

1.0 INTRODUCTION

Poultry production is faced with the challenges of meeting the energy demand of fast growing strains of broilers (Tabeidian, 2010). Poultry production presently occupies a place of pride among livestock businesses due to its rapid monetary income (Folorunsho and Onibi, 2005). According to Ezeibe *et al.,* (2014), poultry production has become an important aspect of the Nigerian Agriculture due to its dual purposes of supplying the populace with meat and egg as well as serving as a valid means of earning regular income. Over the years, the cost of producing broilers is on the increase and this has led to closure of several businesses while young entrepreneurs are not encouraged (Skinner *et al*., 1992). To obtain the best possible productivity in poultry birds, the protein, energy ratios of the ration must be balanced. This is because one third of the cost incurred in poultry (egg production, broiler) production is expended on energy supply (Skinner *et al*., 1992, Ezeibe, 2010). In a bid to augment energy needs of broilers, farmers have devised means of supplementing diets with oils

Dietary fat plays an important role in poultry nutrition. Fat contains at least twice the available energy of carbohydrates and protein (Baião and Lara, 2005). Dietary fats enhance the absorption and utilization of fat-soluble vitamins, increase the palatability of rations, reduce pulverulence, improve the efficiency of the energy consumed (Chwen *et al*., 2013) and reduce the rate of passage of digesta in the gastrointestinal tract, which gives room for adequate and efficient absorption of the nutrients present in such diets (Baião and Lara, 2005). Dietary oils (DO) have been used in compounding feed in poultry due to its high energy value, and because they are required for the proper functioning of the central nervous system and muscular activities. Different dietary oils and fats in poultry diets are often used indiscriminately to spare the high cost of maize without recourse to their

chemical composition which could influence the performance and productivity of chickens (Mossab *et al*., 2000). According to Zambiazi *et al.* (2014) fats and oils are classified as lipids; and are chemical substances of distinct characteristics. This group differs in solubility for solvents that are non-polar such as benzene, ether and petroleum ether, among others, with their heterogeneity occurring in biological material. Hong *et al.,* (2012) noted that lipids with high saturated fatty acid values occur as solids at room temperature and those with higher values of unsaturation occur as liquids (oils).

Mohammed and Horniakova, (2012) observed feeds supplemented with tallow resulted in elevated saturated fatty acids (SFA) in thigh muscle, belly fat pad and breast muscle of broiler chickens compared to those on vegetable oils. Jeffri *et al.,* (1997) also noted that birds on soyabean oil-supplemented diets had elevated polyunsaturated fatty acids (PUFA) in their carcass compared with those on beef tallow. However, Senkoylun *et al.* (1991) reported that the accumulations of fatty acids are a direct result of dietary fatty acid absorption.

The inclusion of oil in poultry diets had improved performance than in poultry birds fed diets without oil (Moura, 2003). Benefits of using oils in poultry comprise reduction in losses of nutrient, ease of absorption and breakdown of lipoproteins, substantial quantity of required fatty acids is obtained and low heat related to sugars and proteins, enhanced palatability of feed, reduced particle, which helps keep an even blend of each portion of the diet (Leeson and Atteh, 1995).

Dietary oils are rich in fat soluble vitamins like vitamin A, D, E and K. Animals kept in total confinement as is the case of modern poultry and pig without exposure to sunlight will require a dietary inclusion of vitamin D. In poultry, vitamin D_2 is transformed to D_3 being the absorbable energetic form. Fat soluble vitamins have a crucial role in calcium digestion with their involvement in the control of calcium absorption and bone mineralisation. The prevalence of locomotion problems in broiler chickens of rapid growth has become a major concern in the poultry market (Almeida Paz *et al*., 2010). The most occurring skeletal defect in broilers fed nutritionally balanced diets is angular bone deformity which occurs as early as one week post hatch and could be visible at four weeks (Julian, 1998, Ruttanavut and Yamauchi, 2010). A critical consequence of this deformity is crippleness and reduced access to feed, water

and rejection during meat processing. The primary cause of angular bone deformity is rapid growth with insufficient time for proper bone alignment and remodeling of the distal tibio-tarsus (Thorp *et al*., 1995). Bone immaturity and heavy breast muscle weakens ligament strength, and these symptoms are typical of spondylolisthesis as reported by Julian (1990).

Zambiazi *et al*. (2014) ascribed higher percentage values to unsaturated fatty acids in the glycerides of vegetable oils. Increased levels of saturated fatty acids are advantageous in oil stabilisation, but detrimental to livestock nutrition. Adedokun and Adeola, (2013) reported that oil utilisation in broiler chickens is directly influenced by the quantity of calcium in the diet, it also determine the extent of digestion of oils, as fatty acids has a way of forming soluble or insoluble soaps in the gut lumen by binding with minerals, such as calcium, phosphorus. Shafey and McDonald (1991) reported increasing production of excreta soaps due to high level of dietary calcium. Suitable levels of dietary calcium and phosphorus resulted into favourable feed conversion ratio as well as increased gain in weight in birds compared to those with calcium shortage (Leeson and Summers, 2000). Also, dietary fat could affect mineral metabolism and reduce broiler performance (Shastak *et al*., 2012). Formation of soluble soap may render the fatty acids and Ca unavailable for absorption (Hosseini- Vashan, 2010). Atteh and Leeson (1983) reported that a large percentage of saturated fatty acids in chicken faeces was present as unutilised soap in contrast with that observed with unsaturated fatty acids. This can jeopardize the energy value of the fat and interfers with the bird's mineral retention, bone and eggshell quality.

Calcium has important biological functions and must be provided in adequate amounts. Inadequate Ca intake may affect bone mineral content, muscle function and other body mineral functions (Peters and Mahan, 2008). Intestinal mucosa plays an important role as the site of nutrient absorption in the small intestine (Julendra *et al*., 2012).

Dietary oils are the main sources of essential fatty acid which cannot be synthesised by the birds and are supplied in feed for improved performance and to obtain energy for their metabolic activities (McDonald *et al*., 2002).

However, attention has been driven towards the nutritional and health consequences of oil inclusion on poultry production. Diets with increased energy density have significantly improved growth and feed efficiency (Hosseini-Vashan *et al*., 2010).

Although, there is controversy on the right amount and type of dietary fats and oils to be incorporated in broiler ration for improved performance and meat quality (El-Deek *et al*., 2005). The degree of saturation in a fatty acid is inversely proportional to the extent of its digestibility and the degrees of unsaturation which has effect on digestion (Mohammed and Horniakova, 2011). Higher levels of fats not only cause indigestion but they form insoluble soaps which causes Ca deficiency and unavailability of Ca despite the levels of Ca in the diet (Jeffri, 2010).

The DO also has effect on digestibility and absorption of carbohydrate, proteins and minerals (Leeson, 1993). The outcome of some fat digestion is in the carcass through the increased abdominal fat digestion and result in lack of demands of such meat by consumers. Increased mortality rates, feed conversion ratio, indigestibility, lower feed intake, lower body weight gain which endangers the life of the bird and reduced the income of the farmer. Therefore, it becomes important to identify some vegetable oils of nutritive importance, their level of inclusion, digestibility to broiler growth and optimise their uses in broiler chicken production.

1.2 Justification

There have been concerns on the need for optimum productivity in poultry production in Nigeria. Efforts are aimed at producing maximally at a reduced cost, and dietary manipulation is one of the adopted mechanisms. Dietary oils have high caloric value, supplies twice the energy obtained from carbohydrates and the heat increment associated with metabolism is low compared to cereals. However, oils are used indiscriminately to spare the high cost of maize without recourse to their chemical composition and the likely effects such inclusion levels could have on performance and carcass traits. There are controversies on oil utilisation and digestion in chickens which are largely affected by the diet, cereal type, dietary calcium content and the fat charcaterstics. Hence, the need to assess the effects of dietary oils and varying dietary calcium levels on performance and calcium retention in broiler chickens.

1.3 Objectives of the study

1.3.1 General objective

This project was aimed at studying the effects of dietary oils and varying dietary calcium levels on performance and calcium retention in broiler chickens.

1.3.2 Specific objectives

The specific objectives were to:

- Determine the physico-chemical characteristics of selected dietary oils
- Investigate the effects of varying levels of dietary calcium and oils on performance, serum biochemical indices, and calcium retention in broiler chickens
- Investigate the effects of oil types and dietary calcium levels on carcass attributes, ileal Ca and P digestibility in broilers

CHAPTER TWO

LITERATURE REVIEW

2.1 Energy requirements contributing to improved growth of broiler chickens

One of the objectives of poultry prodution is to feed the chickens with a balanced diet at least cost and also generate products that will attract premium prices in order to maximise profit (Lopez and Leeson, 2008). Several factors such as genotype, diet composition, digestible nutrient content, energy to protein ratio, feed form, feed processing, environment, and disease could affect the cost of production and poultry product quality through influencing feed intake, body weight gain and feed conversion ratio (FCR) (Leeson and Summer, 1991). Dietary management of energy intake has been reported to decrease the cost of production and improve product quality to a greater extent than the abovementioned factors (Ferket and Gernat, 2006). However, most energy feed ingredients that will help in achieving improved performance, health, reduced production costs and improved product quality in poultry production are continuously becoming scarce and expensive for use in broiler production due to competition for available energy sources used by industries for biofuel and as food for humans (Leeson and Summer, 1996). Feeds that provide the basic nutrients which help to achieve quality broiler carcass yield accounts for over 70% of the overall cost of poultry production, with energy sources being the largest in terms of quantity (40–70%) and invariably the most expensive (Vander Klis *et al*., 2010). The importance of dietary energy in poultry feeding cannot be over-emphasised because increasing or decreasing the dietary energy has been reported to affect feed intake in addition to promoting or undermining efficient feed utilisation and growth rate (Ghaffari *et al*., 2007). Singh and Panda (1992) concluded that birds usually eat with the aim of satisfying their energy requirement, and once this aim is

achieved, the birds will stop eating irrespective of the fact that other key nutrient requirements such as protein, minerals, and vitamins have not been met. (Summers *et al*., 1992).

Poultry require a stable supply of nutrients in their diets to obtain the needed energy, protein, essential fatty acids, minerals for maximum growth (Daghir, 1995). These nutrients are obtained through the digestion of natural feedstuffs and essential amino acids like lysine, methionine, threonine that are included as supplements. The level of energy released by a diet is a determining factor in feed intake. Once there is a change in the energy supplied by the feed, feed consumption will be affected and it must be adjusted to meet up with the required intake (NRC, 1994). Due to this, energy level of the diet is the first consideration while formulating feed for birds. The energy requirement for different breeds of poultry to meet up with their daily requirement is different and failure to meet up with this requirement has a negative impact on their production performance (Daghir, 1995).

Energy and protein are the second most important feed constituents after water and are needed to maintain health, growth, and production. This explains why energy and protein sources are the most important feed ingredients for poultry feeding. Oilseed cakes and animal protein meals are considered as secondary sources due to their substantial energy content (Perry *et al*., 2003). Cereal grains provide 60–70% of dietary energy for poultry, while other energy and protein sources supply the rest. Although the interaction of protein sources with the main energy sources influences the overall energy supply and utilisation (Carre *et al*., 2013).

Chicks acquire a net amount of energy about 60% from the metabolisable energy of proteins, 75% from carbohydrates and 90% from fats (Scott *et al*., 1982). It is clear that chicks could effectively utilise the metabolisable energy donated by fats. Baiao and Lara (2005) observed that dietary oils improved fat digestibility in broiler chicks at starter phase and enhanced performance. The net availability of metabolisable energy obtained by chicks from corn oil was almost ten percent higher than from carbohydrates. Crude soybeans and corn oil contains higher percentage of unsaturated fatty acid and more predominantly, linoleic acid. Cook *et al.* (1993) further described the conjugated isomers of linoleic acid as active in curtailing slow rate of growth, avoiding the building up effects of immune stimulus.

2.2 Importance of dietary protein and amino acids

Amino acid (AA) is the building block of proteins which is needed for optimum growth, egg production, adaptation to the environment and performance. Some amino acids are termed essential, needed for metabolic needs in the body and cannot be synthesised by the body but added up into the diet (Ravindran and Bryden, 1999). Essential AA are affected by several factors which include the health status of the animal, the sex, breed, age, physiological status of the animal and the environment (El-Yamany, 2008). To obtain a lean meat, poultry requires lysine and methionine for increased egg production and growth.

For broiler chickens, diets are often formulated to contain 22% protein for the starter feed and 19% for the finisher feed, with a metabolisable energy value in the order of 3.3 ME/Kg. Chickens may respond differently to the increased protein level in the diet, depending mainly on the protein quality and the amino acid profile thereof. With low quality protein having inadequate and/or imbalanced amino acids, increasing dietary protein in this case will have no effect on performance in terms of growth, feed efficiency and carcass traits, but may rather lead to high mortality and leg problems, particularly in the finishing phase. Addition of the first limiting amino acids in this case will, therefore, be necessary and will result in an increased productive output in the bird (Adedokun *et al*., 2016).

2.3 Fats and fatty acids

Chicks require a net amount of energy, which are obtained from various feed ingredients. Fats and oils are collectively known as lipids. They provide significant amounts of energy to poultry diets, but there is a large variation in composition, quality, feeding value, and price. These notwithstanding, they are regularly used in poultry feeds to satisfy the energy need of the animal as lipids have more than twice the amount of ME than carbohydrates or proteins per kg weight. However, they are normally included at a maximum level of 4–5%. The commonly used types of fat in poultry diets include tallow, poultry fat, feed-grade animal fat and yellow grease. Animal fats provide an average ME of 8850 kcal/kg for poultry. Similarly, oils have a high content of energy, the average ME content of different types of vegetable oils ranging between 8300 and 8975 kcal/kg. The commonly used oils in broiler diets are

soybean oil, canola oil, and palm oil. Besides the concentrated energy, including fats and oils in poultry diets improves the physical traits and palatability of diet, increases pellet durability and enhances the essential fatty acid contents of the diets, especially linoleic acid (Walker, 2011)

Proteins supply 60% metabolisable energy, 75% from carbohydrates and 90% from fats (El-Yamany, 2008). Increased level of calorie is obtained from fat, that is why it is incorporated in poultry diet to obtain the needed dietary energy concentration. The need for essential fatty acid is the main reason for oil addition into the diet of poultry birds. It is clear that chicks could effectively utilise the metabolisable energy supplied by fats. Baiao and Lara (2005) observed that dietary oils improved fat digestibility in broiler chicks at starter phase and enhanced performance. The net availability of metabolisable energy obtained by chicks from corn oil was almost ten percent higher than from carbohydrates. Crude soybeans and corn oil have higher levels of USFA and more predominantly, linoleic acid. Zanini *et al.* (2006) further described the conjugated isomers of linoleic acid as active in curtailing slow rate of growth, avoiding the building up effects of immune stimulus. Zollitsch *et al.* (1997) reported that broiler chicks from day old utilise soya oil included in diets at 3.5% effectively for low fat digestibility.

Surai *et al*. (2003) observed that acid oil and corn oils are rich in carotenoids, which are pioneers for vitamin A synthesis and are necessary for modifiable cell growth and bone metabolism. It also affects both antibody production and T-lymphocyte Pmultiplying responses, where an insufficiency could lead to reduced growth rate. The energy of feed must be such that it permits for satisfactory nutrient density. The capability of the bird to eat and meet energy requirement is influenced by the competence of the diet (Leeson and Summers, 1997). At reduced energy concentration, chickens do not meet their calorie needs and at an increased concentration, take in excess of the essential feed for growth and production thereby becoming inefficient (Leeson and Summers, 2000). Energy is needed for the maintenance of all normal body function and the quantity needed for production and growth is obtained from the diet. Energy supplied in a diet determines the rate of feed intake because poultry birds eat to satisfy the needed energy for their metabolic activities (Krejci-Treu *et al*., 2009). The energy content of feed is usually expressed as kilocalories per gram or per

kilogram. According to NRC, (1994), gross energy values of feedstuffs (kcal/g) are determined by the relative amounts of the 3 major classes of organic nutrients present; carbohydrates, 4.1, proteins 5.7, fats (lipids), 9.4. Thus, feedstuffs high in fat releases more energy than those feedstuffs with only carbohydrates. The gross (total) energy of feed is a perfect index of the volume of energy available to the animal because of the alterations among feeds in the rate of use by an animal. Fatty acids affect the quantity of fat deposited in the body of chickens in various ways. Diets containing Polyunsaturated Fatty Acid (PUFA) cause lower fat deposition than diets containing similar amounts of SFA. Chen and Chiang, (2005) replaced tallow by soybean oil (SO) in broiler diets and observed lower abdominal fat pad (AFP) deposition. Senkoylun *et al.* (1991) found that high levels of a combination of sunflower oil (SFO) and soybean oil in replacing tallow also resulted in lower AFP and lower body fat. Lima (1997) reported that saturated oils resulted into higher AFP deposition than unsaturated oils although they had much lower metabolisable energy.

2.4 Vitamins

Vitamins are essential organic nutrients to animals and participate as cofactors in many metabolic processes acting in more than 30 cellular metabolic reactions (Asensi-Fabado and Munné-Bosch, 2010). In this way, they are essential for growth, productive performance, health, survival and reproduction of broiler chickens. Because they are not produced in adequate amounts, it is recommended to provide them in the diet. However, depending on the animal species and the environment, some can be synthesized. Vitamin D, for example, can be synthesized when the animal is exposed to the sun, while vitamin C may have its guaranteed levels if the animal has the gulonolactone oxidase. In turn, niacin is derived from tryptophan, and choline from methionine (Bertechini, 2012). Therefore, the vitamins should be supplemented in the diet at small amounts compared to other ingredients, and their deficiency cause several problems for the development of animals.

Vitamins are classified into two: fat soluble vitamins (A, D, E and K) and water soluble vitamins (B and C). All other vitamins are included in the diet except for vitamin C which can naturally be synthesised by the bird (Rose, 1997). Vitamins are mediators of all biological routes in the body and are not energy sources.

Vitamin E is one of the vitamins supplemented in the diets, at different levels, as it is essential for the integrity of reproductive, muscular, circulatory, nervous and immune systems of the animals (Habibian *et al*., 2014). It also functions as free radical scavenger and lipid antioxidant thereby reducing meat quality deterioration (Hsu and Guo, 2002). The inclusion of vitamin E in feed for broiler has resulted in positive effects on growth performance when supplemented at higher levels during heat stress (Souza *et al*., 2011). In addition, it can be demonstrated that an improvement in carcass and cuts yield, quality of meat products, reducing the peroxidation of membrane lipids, and increased activation of the immune system (Hashizawa *et al*., 2013). For being of soluble nature, vitamin E participates actively in the structure of organic compounds because it is situated at the membrane level, minimizing the peroxidation of fatty acids and phospholipid components.

α-tocopherol has the greatest biological activity when compared to other forms of vitamin E, because it has higher absorption, higher deposition in tissues and low fecal excretion (Cortinas *et al*., 2005). Excess of α-tocopherol and its analogs are extensively metabolized before excretion. This suggests that the organism tries to maintain a certain level of vitamin E by selective retention of certain amounts of α-tocopherol (Zingg, 2007).

Vitamin E is a component and natural antioxidant of cell membranes and has been considered almost exclusively associated only with the reproductive system of animals. However, currently, it is known that this vitamin modulates inflammatory signaling, regulates the production of prostaglandins and leukotrienes, minimizes damage resulting from cytotoxic action caused by free radicals in the organism, and improves the phagocytic activity of macrophages in broiler chickens (Leshchinsky and Klasing, 2001).

In poultry, the absorption of vitamin E occurs in the non-esterified form in the small intestine attached to chylomicrons and released via the hepatic-portal system associated with very low density lipoproteins (VLDL), which subsequently dissociate into low density protein (LDL) (Liebler, 1993), similar to the lipid digestion. Esters are extensively hydrolyzed in the gut wall, and the free alcohol is absorbed by intestinal vessels and transported via the lymph to the circulation (McDowell, 2000). Only the

alcoholic part is assimilated by the animal. The LDL molecule carries the major portion of vitamin E, and then performs its exchange with high density lipoprotein (HDL) until the latter reach the other tissues, such as liver, muscle, and especially the adipose tissue (Burton, 1994).

Thus, the fat soluble vitamins are called as growth vitamins (Bou *et al*., 2009). Vitamin D is a fat soluble vitamin as well as a steroid hormone, needed by poultry. It has a crucial function in the intestinal absorption of Ca which is mediated by vitaminD_3 metabolite. Also, β carotene and α- tocopherol have a deep consequence of reducing heat load in broiler chickens. Under tropical conditions, acceptable quantities of dietary fat is to be included in broiler diet to stop translation of dietary starches to fatty acids (Lehninger, 2000). Different sources of fats and oils influence their metabolisable value, physical and chemical properties. Lopez-Ferrer *et al*. (2001) attributed poor saturated fatty acid absorption to intestinal thickness and this could result to reduction in transport of emulsion droplets and fatty acids in the gastro intestinal tract (GIT). Also, saturated fats are known for their non-polarity and incapability of micelles formation for proper emulsification (Senkoylun *et al.,* 1991). The endogenous lipid produced in the structure of bile fused to dietary lipids are not adequately broken down and absorbed. The unabsorbed portion passes beyond the ileum and are wasted. Crespo and Esteve-Garcia (2002) discovered that 10% olive oil and lin seed oil inclusion had lower abdominal fat pad deposition, and low level of plasma insulin than those on tallow supplemented diets.

2.5 Carotenes

Carotenoids act as antioxidants but some are also vitamins with essential biological functions (Arczewska-Włosek, 2011). For example, β-carotene and other pro-vitamin A carotenoids are converted into retinol, an essential component of the visual pigment rhodopsin that also helps to maintain epithelial and immune cells, and into retinoic acid, a coregulator of developmental gene expression (Jlali *et al*., 2012). Vitamin A and carotenoid metabolism in chickens is closely related to the equivalent process in humans, so chickens are also susceptible to vitamin A deficiency with similar symptoms (Pretorius and Schönfeldt, 2013).

Carotenoids represent one of the most widespread groups of natural pigments. They are synthesized de novo by plants, but they are present in animals, where they

accumulate either unchanged from the diet, or metabolically modified. There are over 1100 known carotenoids (Yabuzaki, 2017). The characteristic carotenoid pattern in birds is the accumulation of xanthophylls (i.e. carotenoids containing at least one oxygen atom in the molecule), and the almost complete exclusion of carotenes (oxygen-free carotenoids). Plant carotenoids are the primary dietary source of provitamins A, with β-carotene as the best-known example. Others include α-carotene and cryptoxanthin. Carotenoids with high vitamin A activity have low pigmenting properties (Hencken 1992). The essential structural requirement in the provitamin A molecule is one unsubstituted ring attached to an intact conjugated polyene structure, thus β-carotene with two essential structural units is the most potent provitamin A. The main site of the conversion of β-carotene to vitamin A is the intestinal mucosa and two enzymes are involved: oxygenase which splits the molecule in the centre to yield retinal (vitamin A aldehyde) and retinal reductase that converts retinal into retinol. Retinol and metabolites such as retinoic acid play major roles in vision, immune and brain functions, tissue remodelling and metabolism (Brossaud *et al.,* 2017). It has been shown that the absorption of lipophilic vitamins requires to be combined with bile acids and lipid to form micelles (Silva and Furlanetto, 2018). Presumably, also intestinal absorption of carotenoids in poultry may be enhanced by the presence of fat in feed mixtures.

Oil palm has been reported to contain a large quantity of carotenes in the leaves and fruit. About 500-700ppm of carotenes are present in unrefined palm oil that are between 24 and 42% and 50-60% β-carotenes with other carotenes in low quantities. Processing of palm oil reduces and sometimes destroys the carotene content of oils. Carotenes are a natural source of vitamin A, a potent antioxidant against autoxidation and photooxygenation (Tan, 2012), palm oil has a balanced fatty acid composition in which the level of saturated and unsaturated fatty acids are almost equal with 50% saturated, 40% monounsaturated and 10% polyunsaturated fatty acids. Palm oil contains ca, 3% of free fatty acids and 1% of other minor components. The minor constituents of palm oil include carotenoids, tocopherols, sterols, phosphatides, triterpenic and aliphatic alcohols. Among them, the most important compounds are carotenoids and tocochromanols (tocopherols and tocotrienols). Phoon *et al*. (2018) reported that crude palm oil contains 500–700 ppm of carotenoids and 1000–1200 ppm

tocochromanols. Carotenoids of palm oil are mainly in the form of alpha- and beta-carotenes, the precursor of vitamin A. The presence of these carotenes plays an important role in oxidative protection to the oil. On the other hand, the major portions of total tocochromanols in palm oil are alpha-tocopherol and gamma-tocotrienol. These compounds are also antioxidants and provide some natural oxidative protection to the oil. It is obvious that the combination effects of properties of carotenoids, tocochromanols and high portion of unsaturated acids give palm oil a higher oxidative stability compared to many other edible oils (Tehlah *et al*., 2017).

2.6 Mineral concentrations in poultry diets: Implication on nutrient absorption

Minerals are important for the maintenance of the acid-base balance of the body, the skeletal framework and overall functioning of the body system (Hutton *et al*., 2014). Macro minerals are needed in large quantities in the feed among which are sodium, potassium, chloride, sulphur, calcium and phosphorus are the most abundant. They are needed for general maintenance, eggshell quality and a good skeletal development (Vignale *et al*., 2015). Calcium and P must be maintained in the ratio of 2:1 in order to optimize their uptake for optimum efficient growth in growing bird. However, the requirement for calcium in laying bird is high (13:1) because of good egg shell quality (NRC, 1994).

Identification of the site of absorption of mineral nutrients like calcium and phosphorus is important in understanding the dynamics of digestion. Burrell *et al.* (2004) examined the consequence of inclusion of dietary fat and different calcium levels on broiler chicken's performance and reported a decrease in the overall feed consumed by the broiler chickens on 1.5% Ca compared to those on 0.93% Ca. According to NRC (1994), best performance in broiler chickens were observed at ratio of 2:1 calcium to phosphorus. A broader Ca: P adversely affects the utilisation of both minerals, and when the rate of inclusion is low, mineral retention was achievable (Hemme *et al*., 2005). In turkey poults, the proficiency of phosphorus utilisation was observed to be moderately influenced by Phosphorus concentrations but Ca utilisation was adversely influenced by the quantity of Ca in the diet. Reports have suggested that

increased calcium levels could have a negative impact in utilisation of fat, nitrogen and metabolisable energy in broilers (Shafey and McDonald, 1991).

Trace minerals are supplemented to avoid deficiencies that cause disturbances in metabolic processes including loss of appetite, lower performance, impaired immune system, and reproductive disorders (Van der Klis and Kemme, 2002). Trace minerals include manganese (Mn), copper (Cu), and zinc (Zn) which are essential in various body functions, optimal growth and health (Richards *et al*., 2010). Trace minerals are involved in several physiological, digestive, and biosynthetic processes within the body. They are cofactors in many enzymes and act as catalysts in enzyme system and participate in immune defense system and hormone secretion pathways (Dieck *et al*., 2003). Trace minerals influence bone development, feathering, growth, and the rate of feed consumption (Nollet *et al*., 2007). Traditionally, inorganic trace minerals are supplemented to poultry diets to provide levels of minerals that allow the birds to reach its genetic growth potential and prevent clinical deficiencies (Miles *et al*., 1998, Aksu *et al*., 2011)

According to Dieck *et al.* (2003), minor minerals like copper, iron, manganese and zinc are crucial in broiler development, because they participate in physiological, intestinal and biosynthetic routes in the body. They operate as mixtures in enzyme activities and are component of proteins involved in intermediary breakdown and immunity. Bao *et al.* (2009) indicated that these minor minerals are transported in their inorganic salt form as oxides to supply minerals and promote hereditary growth potentials in birds. Natural sources of minerals are being protected from insoluble complexes with secretions from the gastrointestinal tract (GIT). However, when trace minerals are broken down, biological molecules aid their uptake and their utilisation. Minor minerals are compulsory in the routine operation of every biological procedure in an animal (Tamin *et al*., 2004).

2.7 Organic minerals in poultry

Organic minerals could be defined as any mineral that is bound to an organic compound irrespective of the bond type existing in the minerals. Amino acids and carbohydrates are key constituents that make up an organic mineral. Important metals such as Zn, Cu, Fe, Mn form coordinated bonds that are slightly stable when digested

and absorbed. Some metals bound to organic ligands can breakup during digestion, however, real covalent bonds will not coordinate bond because they have a metal bound to an organic ion through coordinated bonds (Shastak *et al.*, 2012). Effective utilisation of organic mineral depends on the ligand which aids its use and absorption in the body. They are first digested then absorbed due to their long chain nature

Poultry products are high in demand for consumption, and supply is not commensurate with the demand. High level of sophistication is adopted as well as nutritional advances with the addition of additives to the diets to enhance the efficiency of birds to obtain market weight within 6 weeks, for example prebiotics, probiotics and organic acids (Jahanian *et al.*, 2008). Carbohydrate and proteins are the most utilised tools in organic mineral concentrations (Surai *et al*., 2003). During its absorption and digestion, organic minerals may present physiological effects that enhances the immune response in large scale production, trace minerals are included as inorganic forms (oxide salts) and they can be prevented from being active by interfering substances in the diet (Bao and Chot, 2009). They are usually included above the NRC recommended rate in poultry diets due to its ease of absorption and are relatively retained because they could be easily excreted to the environment. To prevent this excessive loss, organically chelated minerals are usually added to the diet that aids uptake of the minerals at the upper section of the GIT which has greater bioavailability of organically complexed minor minerals and could be included at a very low rate to prevent excretion (Bao and Chot, 2009).

2.8 Total Tract Digestibility

For over several years, assessing phosphorus (P) availability in feed ingredients with respect to monogastrics, especially poultry have been on different scientific approaches (Rodehutscord and Dieckmann, 2005). Phosphorus is a critical nutrient for animals. There is an increasing interest in improving the utilisation of dietary P for animals because of concerns over environmental pollution through excess P excretion, depletion of nonrenewable global inorganic phosphate deposits, and unpredictable prices of inorganic phosphate supplements. However, measurement of digestible P may be the preferable method to assess P availability for poultry (Rodehutscord, 2009). Published data on apparent or true digestibility values of P in common feed

ingredients for pigs are available (Fan *et al*., 2001; Bohlke *et al*., 2005). However, 3 approaches have been used, namely regression analysis, direct method, and substitution method, to estimate P digestibility (Fang *et al.,* 2007).

Corresponding data for poultry, however, are limited. Dilger and Adeola (2006) estimated the true P digestibility of soybean meal for broilers using the regression method where soybean meal was used as the only dietary source for Ca and P. Wu *et al*. (2004), using the direct method where the test ingredient was used as the sole source of dietary P, determined the apparent ileal digestibility of P in sorghum, wheat, and corn. Leytem *et al*. (2008), using the direct method, measured the apparent ileal and total tract digestibility of P in corn, barley, and oats for broilers. Presently, available P in feedstuffs is generally referred to as nonphytate P (NRC, 1994), which is defined as the portion of P that is not bound to the phytate molecule.

However, available P and nonphytate P are used interchangeably, although studies have clearly demonstrated that nonphytate P is not totally available while phytate P is not totally unavailable to the animal (Angel *et al*., 2002; Coon *et al*., 2002). Retainable P, on the other hand refers to the P that is retained in the body. However, increasing dietary concentrations of nonphytate P result in increased levels of plasma inorganic P, and once the physiological threshold is reached, the excess P is eliminated via the urine. Studies by Manangi and Coon (2006) suggest that, for 40- to 50-day-old broilers, the critical threshold range for dietary nonphytate P is between 2 to 3 g/kg. Digestibility values of P for pigs are usually determined over the total tract (Fang *et al*., 2007; Stein *et al*., 2008; Akinmusire and Adeola, 2009), and this approach is workable because fecal samples can be collected without urine contamination.

2.9 Factors that inhibit organic mineral absorption

Macro and microminerals have long been known to directly or indirectly influence poultry gastro-intestinal health, metabolism and growth performance. Trace minerals like Fe and Cu, can act as pro-oxidants, reducing the stability of vitamins and enzymes and promoting oxidation of lipids (Cohen, 2014). Inadequate mineral supplementation during the growth phase of birds results in an imbalance in mineral homeostasis and poor development of bones (abnormal bone calcification). However, excess calcium (Ca) may act as an antagonist, making it difficult to absorb trace minerals such as iron

(Fe), copper (Cu), zinc (Zn) and other minerals such as magnesium (Mg), sodium (Na) and potassium (K) (Waldroup, 1996). High Ca levels in broiler chicken feed increase the need for phosphorus (P) because Ca interferes with phosphorus absorption. Ca and P form complexes in the intestine, making P less available hindering the absorption of phytin phosphorus by the bird (Ravindran *et al.*, 2006). They are usually supplemented in the form of inorganic salts, such as sulphates, oxides and carbonates, to ensure healthy development and greater productivity.

Finely ground $CuSO_4$ results in increased rates of fat oxidation in the feed, as compared to coarsely ground $CuSO_4$ (Miles *et al*., 1998). Zinc and Cu are acknowledged to be important modulators of the endogenous mechanisms of defense against infections, inflammations and oxidative stress (Bortoluzzi *et al*., 2019). Generally speaking, the chemical form in which minerals are presented in the diet, together with several other factors such as the total mineral concentration in the feed, particle size, feed processing, strain and age of the animal, and the potential of the mineral to interact with other dietary components have been proved to influence mineral availability and, as a consequence, their effects on the physiology of animals (Miles *et al*., 1998)

Trace minerals supplementation is not novelty to poultry nutrition, but producers are now feeding them at higher levels to increase birds' intestinal health. When minerals from inorganic sources are fed and reach the upper parts of the gastro-intestinal tract (GIT) they tend to dissociate due to the low pH and be absorbed (Bortoluzzi *et al*., 2019). In the lower GIT, however, the higher luminal pH increases the interaction between mineral cations and other dietary components (Mwangi *et al*., 2017), leading to the formation of insoluble complexes with much lower availability to animals. However, higher rates of mineral absorption in broilers have been described in the duodenum rather than in more distal segments of the intestine (Van Der Klis *et al*., 2002).

The widest known and studied example of these insoluble complexes is probably phytate, originated from phytic acid and its 12 reactive sites (6 strongly acidic, 2 weakly acidic, and 4 very weakly acidic) (Angel *et al.*, 2002). Macro and micromineral cations, particularly Ca, Zn, and Cu, bind readily to phytic acid as pH increases above

4, forming insoluble complexes that not only decrease the availability of minerals, but also the hydrolytic function of phytases (Angel *et al.*, 2002).

Organic or chelated form of microminerals may have higher bioavailability than inorganic sources, mainly because of their different route of absorption and lower interaction with other dietary constituents. Chelated minerals are metallic ions bound to organic substances, such as amino acids, peptides, or polysaccharides that create a stable and soluble molecule with high bioavailability. Chelated minerals show superior absorption than inorganic forms because usually the mineral is absorbed through the path of the organic ligand that the ion is bound, avoiding its interaction with other molecules. The mechanism by which the ligand improves the use of the mineral depends upon the capacity of the ligand to bind to the mineral and its capacity to compete with other ligands, creating soluble complexes with the mineral (Kratzer and Vohra, 1996).

Similarly, a threshold of dietary concentrations of minerals needed to affect *in vivo* P digestibility or PP hydrolysis may exist. For example, results from a turkey poult study where up to 161 mg/kg of dietary Zn was fed showed no differences in apparent ileal P digestibility or PP hydrolysis. Calcium, while on one hand forms weaker complexes with individual phytate molecules, may cause more complications due to the high quantities used in the diet (Tamim and Angel, 2004)

2.9.1 Effect of minerals in poultry nutrition

Antioxidants are vital tools in balancing oxidative impairment and defence in poultry industry. Antioxidants are included in poultry diet and they come in different forms and some are mineral dependent. In balancing the oxidative damage and antioxidative defence in poultry, the anti oxidant capacity of optimizing the nutritional inclusion of anti-oxidants minerals like selenium, Gluthathione peroxidase, iodothironine. The need in the body is minute, however if not met, the anti-oxidant system is conceded in several health challenges. In a living system, natural mineral complexes are formed when food is being digested and nutrients are taken up which leads to the formation of some natural mineral complexes that hinders utilisation of ingested feed. According to Herrick and Ashmead (1993) minerals are categorised into three types based on their function in biological systems.

(a) Complexes which transports and store ion
(b) Complexes that are essential to physiological activity
(c) Complexes that interfere with metal ion utilisation.

Amino acids and other ligands are important as metal binding and transporting agents in the GIT which enhances the uptake of metal ions from the intestine to the mucosal cells.

2.9.2 Effect of minerals on performance

Climate change can cause severe damage in the poultry production. Heat stress is one of the prominent environmental elements which can influence meat quality (Lara and Rostagno, 2013; Wang *et al*., 2017). The high environmental temperature may cause reduced performance and increased mortality. A solution for the prevention of heat stress includes biological (e.g. genetics, thermal conditioning, nutrition) (Daghir, 2008) or keeping technology devices (air conditioning, intensive ventilation, humidification) (Armstrong, 1994; Wolfenson *et al*., 2001) However, housing methods are expensive and not always adequate. Generally, feed additives with direct or indirect antioxidant effects can be used: vitamins alone or with micro minerals. According to several studies (Daghir, 2009; Mujahid, 2011; Sahin *et al*., 2009), vitamins and micro minerals are most commonly used in poultry nutrition during times of heat stress.

In feed ingredients, organic minerals are included at a reduced rate without any adverse effect on the productivity of the animal. When metal specific amino acid was included in a diet, there was a positive influence on feed conversion ratio over the 45th day of the trial than in inorganic mineral, however performance was not influenced by increased addition of organomineral (Burrell *et al.,* 2004)

Jahanian *et al*. (2008) fed broilers with diets containing 100% organic minerals either of Fe, Mn, Cu and Zn and recorded an increased body weight and a good FCR than those fed inorganic minerals. Jahanian *et al.* (2008) reported the advantages of incorporating minerals in diets of livestock as these sources enhanced intestinal utilisation of trace elements by minimising the interference of mediators that produce insoluble complexes with the ionic trace elements. Problems associated with build-up of heavy metals during fertilisation of crop lands with poultry litter, had pushed for minor heights of mineral leftover disposed in the environment. Naturally, complexed

minor minerals supplies another pathway for incorporation, and leads to decreased mineral flow (Lesson, 2003).

2.9.3 Effect of calcium on oils

Dietary oils have been in use to increase the energy value of feed ingredients and meet up with the energy demand of fast growing broiler chickens.

Fat is usually included in the diet of broilers to meet the energy needed because broilers are now produced to get to market weight with the new strains as early as six weeks, thus the energy requirement must be met in such a way that the heat that is associated with metabolism is minimized. Metabolism of fat is twice the energy obtained from carbohydrates and proteins. That is why fat plays a crucial role in poultry nutrition (Baião and Lara, 2005).

The fatty acid profile in a portion of meat sample has effect on the dietary fatty acid profile (Abdulla *et al*., 2015). However, in digestion of fats, free fatty acids produces a complex with calcium and form soluble or insoluble soaps when fats are digested (Heaney, 1990). Insoluble soap formation renders both the fatty acid and calcium unavailable for absorption (Senkoylun *et al.,* 1991). Senkoylun (1991) reported a proportion of SFA in the faeces of chicken that was present as an unutilised soap compared with USFA. Insoluble soap formation reduces the energy value of the oil, reduces the mineral retention of the bird and eventually has effect on quality of the eggshell and bone thus in adding oils to poultry diet, there is the need to know the quantity of calcium to be added to prevent insoluble soap formation and reduces the amount of nitrogen that is excreted into the environment. Calcium has a crucial role in metabolic functions and must be added in sufficient quantities in feeds. Calcium has effect on bone mineralisation, muscle functions and other mineral functions because modern strains of broilers are selected for higher growth rates and high feed conversion ratio and a deficiency could be easily noticed (Pardio *et al*., 2001). However, mineral requirement especially Ca as recommended by several organisations may not support optimal broiler performance in present strain of broilers however, the small intestine is the site of nutrient absorption where the intestinal mucosa being an active site (El-Yamany, 2008).

2.9.4 The use of fats in poultry

Natural antioxidants have special importance in the maintenance of high growth levels, reproduction and immunocompetence in poultry production. Insufficient intake of antioxidants, a high intake of pro-oxidants or both may lead to oxidative stress (Jiao, 2010). Dietary polyunsaturated fatty acids (PUFA) are a group of pro-oxidants known to increase oxidative stress *in vivo* in hens and chickens (Gao *et al*., 2010). A large proportion of PUFA in dietary plant oils increase the requirement for antioxidants (Cortinas *et al*., 2005). Additionally, lipid oxidation has negative effects on meat quality of broiler chickens (Brenes *et al*., 2008). However, an increase in the amount of n-3 PUFAs in foods, especially docosahexaenoic (DHA) and eicosapentaenoic (EPA), may confer greater susceptibility to lipid oxidation, and oxidative deterioration adversely affects the sensory quality of products, including odours or flavours during storage (Gonzalez-Esquerra and Leeson, 2001).

Fat also known as lipid and describes different types of compounds insoluble in water and dissolve in inorganic solvents e.g. chloroform. Lipids play a crucial role in physiology of animals. Lipids that are of nutritive value are triglycerides, phospholipids and the inherent vitamins in the oils (Brindley, 1984). They serve as the principal source of stored energy in an animal with the highest energy reserve compared with other nutrients in the diet. The number of carbon atoms found in sugar is more than that of fats because those inherent in fats are chemically more reduced thus they could release more energy when they are oxidised. Some of the benefits of adding oil to the diet is obtain and absorp the inherent vitamins in the oil, making the feed palatable and increases the utilisation of the energy consumed also enhances good absorption of the nutrients in the feed by reducing the rate of passage of the feed in the gastro intestinal tract (Moura, 2003).

Addition of oil greater than 40g/kg has been reported by Wiseman and Salvador, (1999) to have negative impact on pellet quality. Sanz *et al*. (1999) fed broilers with either sunflower oil and tallow and observed that utilisation of SFA led to accumulation of abdominal fat compared to birds fed soybean oil. Newman *et al*. (2002) also reported that birds fed diet containing 8% beef tallow had a very low feed efficiency compared to those fed sunflower oil. Mossab *et al*. (2000) confirmed that

the palatability of diets containing fat determines feed consumption and better performance.

Common dietary oil used in feed formulations include frying oils, tallow, poultry fat, soybean, corn oil and palm oil. Oils are not only used as energy source in the diet, but also affect caracass qualities (Senkoylun *et al.*, 1991). The inherent α-tocopherol and carotene in fish oil acts as modulators of immune response in pullets fed diet fortified with fish oil compared to those fed corn oil, linseed oil, canola oil and Lard, medium chain fatty acids such as capric acid, caproic acid has been reported to be effective in curbing *Campylobacter jejuni*. They are sources of essential fatty acids and lipophilic vitamins (A, D, E and K), enabling the birds to derive satisfaction from the feed due to the reduction in the speed of transporting the feed during digestion and enhances the texture, taste and smell of the diet (Lehninger, 2000).

2.9.5 Lipids

Lipids are groups of compounds that dissolve in organic solvents but insoluble in water, thus leading to alkaline hydrolysis into alcohol and some salts of the component fatty acids. On this definition, lipids could be classified into saponifiable and unsaponifiable groups. Saponifiable groups could be simple lipids and those on the unsaponifiable fraction are compound lipids that are alcohols (De witt *et al.*, 2009).

Simple lipids: They have different alcohols and are mainly fat and oils with glycerol, while waxes are fatty acid esters with moderately high molecular weight. A typical example is the medium chain fatty acids (MCFA). MCFA has a rare property that makes it widely used in treating some digestive disorders, because they could be easily absorbed without bile and this makes them a good source of energy. In a case of bile insufficiency, MCFA are used in feed to prevent malabsorption because they could easily bypass the lymphatic system to be fully absorbed.

Compound lipids: are made up of minerals, protein and carbohydrate with alcohol and long chain fatty acids (LCFA) like phospholipids (Nitrogen and phosphoric acid), glycolipids (carbohydrate) and lipoproteins. The carbon chain of the fatty acid has some influence on digestion and absorption along the GIT. Cholecystokinin is an hormone responsible for the digestion and the absorption of oils, it is activated by the entry of the feed into the duodenum through the stomach and this promotes the release

of bile from the gall bladder that separates the fat into small droplets that aids its absorption (Symersky *et al.*, 2002).

The pancreatic lipase joins with the triglycerides to form several fatty acid molecules that combines to form micelles, which are absorbed into the intestine through the fatty acid transporters. As soon as the micelles arrive the intestine, they are conveyed into the endoplasmic reticulum for chylomicrons formation. The chylomicrons are taking to the bloodstream via the lymphatic system. In the intracellular space, long-chain fatty acids bind to carnitine for transport into the mitochondria for subsequent β-oxidation. In carnitine deficiency it resulted into severe protein malnutrition (chronic malabsorption, retarded growth and death), these long-chain fatty acids cannot be efficiently utilised and instead lead to accumulation of unoxidised fatty acids (gluconeogenesis) (Limketkai and Zucker, 2008).

By contrast, MCFA digestion is rapid and simple. The MCFA do not stimulate cholecystokinin secretion. The MCFA absorption occurs via passive diffusion along the gastrointestinal tract into the portal system bound to albumin. No further packaging or modification of the MCFA molecules is required. Moreover, MCFA are not dependent on the carnitine acyltransferase system for transport into the mitochondria for β-oxidation. This provides the ability for more rapid metabolism (Symersky *et al.*, 2002).

Derived lipids: are derivatives of simple and compound lipids through melting of FA, glycerol, fat soluble vitamins and terpenoids. Sterols are saponified but are soluble in lipid solvents. They are categorised as lipids based on their solubility. Cholesterol has a crucial role in membrane structure, being a precursor for steroid hormone production and bile acids.

2.9.6 Lipids In Swine Feeding

In rescent times, the weaning of pigs are now between 15 and 28 days after birth, for optimum productivity (Dierick and Decuypere, 2003). However, the GIT is not fully developed and this predisposes them to some digestive disorders. The sudden change in feed consumed and the environment when weaned increases the inability to absorb nutrients in feed efficiently and decreases feed intake (Liu *et al.,* 2000). This inturn

leads to insufficient energy intake of weaned pigs and could lead to stunted growth and development. Weaned pgs require a high energy diet to prevent stunted growth.

Long chain fatty acid (LCFA) is included in pig's diet as energy a source and essential fatty acids. Due to high content, a reduced fraction of the feed is given to the animal to reduce regurgitation, still birth, aspiration at birth and some GIT infections. Also, Limketkai and Zucker, (2008) reported that LCFA absorption has reduced to about 65% at weaning which partly maybe due to low lipase activity that aids intestinal absorption of digested fat. However, carnitine which is quite responsible for the transportation of LCFA into the mitochondria for generation of ATP require a period of time before they could be synthesized by newly weaned pigs. However, the antioxidant defence may be altered due to the stress involved with the weaning of pigs Chew (1995) reported that Long chain fatty acid are susceptible to attack due to the double bonds in between the carbon atoms. This process causes a lot of damage to internal lipids that should be digested by forming organic free radicals which leads to peroxidation. Thus when formulating feeds for weaned pigs, the digestive system must be placed into consideration to improve digestion along the GIT to reduce the stress associated with weaning.

The MCFA's consists of about 6-12 carbon atoms and esters of glycerol which are obtained from palatable oils such as coconut oil. In comparing LCFA with MCFA, pancreatic lipase acts on MCFA and breaks it down to fatty acid and glycerol, which are absorbed easily by the portal vein then carried into the liver for quick oxidation (Berger *et al*., 1991). Also, MCFA are more stable than LCFA because they are fully saturated with hydrogen atoms. Mutucumarana and Ravindran, (2016) reported that average daily gain, feed efficiency and performance were greater by MCFA addition from 20 to 60 g/kg diet in the first week of life compared to those fed diet containing tallow. Also, Zentek *et al*., (2011) reported that for MCFA diets, energy digestibility was enhanced than LCFA containing diet 98.5% and 93.4% in weanling pigs.

2.9.7 Response of broiler chickens to sources of vegetable oils

Dietary oils are rich in calorie and supplies energy at a lower cost and a good source of fat soluble vitamins (Chowdhury *et al*, (2014). Indiscriminate use of dietary oil has been responsible for unquantifiable losses in poultry industry. Physicochemical

attributes of oils are undertaken to know the identification, purity and quality of a particular oil irrespective of location or sources of origin (AOAC, 1990). It provides an insight into the composition of the oil and how best they could be effectively utilised in livestock nutrition. Oils are one of the daily needs of humans and also a high energy source needed for metabolism. Oil types have a great impact on wellbeing of animals because high intake of trans and saturated fats have been reported to negatively increase respiratory diseases and inflammation (Seyrek *et al*., 2004). In a review by Chowdhury *et al*, (2014), he stated cardiovascular diseases is not in any way related to the intake of saturated fats. It has been reported that daily intake of PUFA in form of fish oils is enough to enhance reduction plasma triglycerides and platelet aggregation.

Oils are generally used in broiler diet as energy source. Some of the benefits of oil inclusion in diets are reduction in nourishment dust, better utilisation of lipoproteins, required fatty acids and reduced heat load during digestion. They also aid incorporation of total carotene, α-tocopherol and calcium. However, its use is limited because of negative effects on pelleting, difficulty in metabolisable energy calculations and rancidity (Fattore and Fanelli, 2013).

Also, vegetable oils with high degree of unsaturation have increased energy levels compared to saturated animal fats. Several oil types are available for poultry birds. They include sunflower, soya beans oil, canola oil, among others. Thacker and Campbell (1994) reported that broilers on diets supplemented with different types of canola oil had better performances than birds on tallow and acidulated soya beans oil. This result ascertains the benefits of incorporating vegetable oils in the diet in place of tallow, because they contain increased level of LCFA. However, Shahyar, (2011) recounted that inclusion of canola oil and poultry fat improved body weight and optimum FE in broiler chickens. Also, Zanini, (2006) noted that canola oil inclusion in the diet of broilers decreased the quantity of lipid of the breast meat and liver. De witt *et al*. (2009) observed a good FE in birds on 6% dietary inclusion of sunflower oil, soy bean and fish oils compared to beef tallow. However, the digestion and utilisation of oils along the GIT and the efficient utilisation of minerals and nutrients has not been fully investigated.

2.9.8 Challenges facing the use of dietary oils in poultry production

The contribution of poultry to meeting animal protein requirement and maintaining the health status of human population cannot be over emphasised as people of low income could be able to afford it (Riaz *et al.,* 2014). However, poultry industry is faced with alarming and increasing human population, outbreaks of diseases and feed ingredient scarcity. Therefore it becomes mandatory to utilise non-conventional feed ingredient and feed additives to augment production in poultry industry (Jongbloed and Kemme 2002). Leeson (1993) noted that lipids among other feed ingredient contained high energy density, and are chemically referred to as triglycerides or triesters of glycerol and fatty acids. They function in protecting the body against excessive body temperature, hormone synthesis and adequate performance of nervous system. Fats are reservoirs for calorie in the body and are in glycogen forms. The major source of essential fatty acid which is not obtainable by synthesis in broiler chickens is dietary fat supplementation and must be provided for improved performance and to achieve more energy to meet up with the needs of fast growing chicks within a short period.

Associated problems of fat utilisation include the adequate amount to be included in rations and digestibility in broiler chickens varies with age and source of fat (Leeson, 1993). Mohammed and Horniakova (2012) stated that the level of saturation is directly related to digestibility and the unsaturation degree in the fatty acid chain increases digestibility. Increased levels of fats leads to indigestion and also prevent the digestibility of other nutrients especially calcium by forming insoluble calcium soaps regardless of the amounts being added in the diets (Tabeidian *et al.,* 2010).

2.9.9 Oxidation of fatty acids and its metabolism

Triglycerides are the major store of fat in the body because the energy yield from a gramme of fat is twice that obtained from protein or carbohydrates (Zanini, 2006). The most effective use of fatty acids is by β-oxidation in the mitochondria to yield energy, though they are reserved in the adipose tissues. However, this process requires carnitine as a means of transportation into the mitochondria. The structure of fatty acid has effect on the rate of oxidation, for instance, LCFA are less oxidized, USFA are easily oxidized while SFA is the least oxidized. Also, oxidation increases with the chain length (Zanini, 2006).

2.9.10 Calcium

Calcium is a mineral that forms about 20% of the weight of the body which is predominant in the skeleton, teeth, plasma in the blood and the some tissues in the body. The main function of calcium is to provide structure and strength which is supplied in form of Ca-phosphate mostly located in collagen and aids blood Ca and bone equilibrum as well as those found in the body fluid giving room for ion exchange. Ca plays a myriad of key roles in cellular biochemistry and physiology of living organisms. Calcium is also the cofactor of many enzymes, and is critical in the maintenance of the electrical probable variance across cellular membranes. Finally, 70% of bone is made up of hydroxyapatite, a mineral composed of different calcium salts.

The solubility of Ca complexes may be very distinct from that of inorganic salts because they depend on pH, considering that one of the most important factors regarding the bioavailability of calcium is its solubility (Heaney *et al.*, 1990) and that the pH of the GIT with time and section, varyies from high acidity in the stomach to low pH or almost neutral in the intestine, pH has a great influence on absorption of calcium. Van der Klis *et al*. (2002) evaluated the equilibrium solubility of five calcium salts (Ca-oxalate, Ca-phosphate, Ca-citrate, Ca glycerophosphate, Ca carbonate) at various pH values and observed that the higher the pH, the lower the solubility. These results suggest that most Ca salts, with the exception of Ca oxalate, are expected to be soluble in the stomach, Ca probably does not stay in solution in the intestine, the main absorption site. The conditions of the small intestine are especially relevant, since insoluble calcium compounds cannot diffuse through the brush border membranes of enterocytes and therefore cannot be absorbed.

The administered marker may be extrinsically added to a single or multiple meals. The absorption extent is calculated by subtracting the amount of marker recovered in faeces from the amount ingested orally. It is very important when using this technique that stool samples are completely collected, otherwise absorption will be overestimated. On the other hand, some nutrients, such as calcium, may undergo endogenous faecal excretion, that is they are excreted into the gastrointestinal tract, after its absorption, and not reabsorbed, leading to underestimated absorption results. Intravenously administering another calcium isotope and analyzing its content in the stools allows the

determination of fecal excretion extent and correction of absorption results. This double stable isotope approach may also be used for determining absorption based on the analysis of plasma or urine samples at specific time points (Taylor and Bushinsky, 2009), avoiding the need of complete faeces collection. Stable isotope techniques are also used to study calcium metabolism, mainly bone calcium turnover and endogenous excretion.

Calcium stable isotopes were firstly analysed by radiochemical neutron activation analysis (Shastak *et al.*, 2012), but mass-spectrometry techniques progressively became the method of choice due to their higher precision. In this field, mass spectrometry atom bombardment and thermal ionisation spectrometry (Jongbloed and Kemme 2002) have been used, but presently, the most employed technique is inductively coupled plasma mass spectrometry. Although calcium isotope ratios measurements by using conventional sample introduction is subjected to interferences of several other elements, technological advances such as mass spectrometry (Sullivan, 1992) have been developed to overcome such limitations.

Ca in the blood regulates several hormone related reactions such as nerve impulse, and blood coagulation because Ca, phosphorus and vitamin D_3 are needed for good bone formation. Majority of Ca present in the body is found in bones and the remaining are involved in different functions in the body such as metabolism and physiological functions. It aids blood clotting, contraction of the muscles and transmission of nerve impulses. Inadequate Ca intake can lead to some defects such as stunted growth, rickets and poor bone formation in broilers. Also, high intake of Ca can lead to reduction in the availability of some nutrients and minerals such as P, lipids and protein (Mutucumarana and Ravindran, 2016).

Recently, the price of inorganic phosphate and environmental P pollution has necessitated the need to research into digestible Ca in feed ingredients, (WPSA, 2013). This is done to certify that Ca and P needed by birds are met and the relationship of Ca and P throughout digestion are taken into consideration. However, the digestibility of these minerals invivo has not been given enough attention.

2.9.11 Calcium sources for poultry

The main source of Ca in feed is the feed ingredients, which is usually low (between 0.02 and 0.06%). Thus inorganic source of Ca is usually added to the feed, they include limestone and dicalcium phosphates, monocalcium phosphate and animal proteins include bone meal and oyster shell to meet up with the requirements (NRC, 1994).

2.9.12 Endogenous calcium losses

Biliary secretions, digestive enzymes, intestinal mucosa are some of the ways in which Ca is transported into the walls of the Gastro intestinal tract of birds (Mutucumarana and Ravindran, 2016). It has been reported that calcium is absorbed across the intestinal wall viua 2 pathways; transcellular and paracellular routes (Wasserman, 2004; Hoendrop *et al*., 2005; Fajita *et al*., 2008). Transcellular active transport occurs predominantly in the upper section of the small intestine, especially in the duodenum and upper jejunum in several animal species including mice, rat and chicknens. About 10% of calcium absorption via this route takes place in the colon of rats (Auchere *et al*., 1998). The transcellular calcium absorption occurs via the epithelial calcium channels from the intestinal lumen into the enterocyte (Peng *et al*., 2003), intracellular calbindin for trans-cystosolic diffusion and the adenosine triphosphate activated basolateral membrane calcium pump (Carafoli, 1991) and Wood *et al.,* (2002) have extensively documented vitamin D_3 is involved in each of these steps compulsory for calcium absorption. Unlike the transcellular pathway, the paracellular pathway is non saturable and absorption occurs throughout the small intestine (Auchere *et al*., 1998). Recently, it was widely believed that calcium absorption in the ileum occurred predominantly via vitamin D_3-independent paracellular pathway and that it is concentration dependent (McCormick, 2002). As reviewed by Adedokun and Adeola, (2013) *invitro* and *invivo* studies did not contradict the abundance of the respective transporters for calcium in the upper segment of the small intestine. The length of the distall jejunum and ileum as well as the duration of time the digesta spends in the jejunum and ileum provides another reason why paracellular absorption of calcium and phosphorus may contribute significantly to the amount of these minerals absorbed. The saturable trascellular mechanism in the small intestine absobs calcium following three steps. The calcium in the lumen enters the enterocyte by channel proteins that are

located in the membrane. These channels increases are open if the luminal calcium concentration is low and the number of channels increases in response to vitamin D_3. calcium is then transported through the enterocyte by calcium-binding protein such as calbinding is delivered to a Ca-Na exchanger, which exchange 3ions to a Ca-ATP that pumps Ca out of the cell. Both the Ca-Na exchanger and Ca-ATPase are located on the basolateral membrane of the enterocyte. The kidney plays an important role in the regulation of calcium by filtering, reabsorption and excreting the calcium in the blood to maintain stable calcium level in plasma (Taylor and Buskinsky, 2009). Calcium is absorbed regardless of the dietary calcium and phosphporus concentration, but if calcium is absorbed in excess of requirement, excess are excreted in the urine (Stein *et al*.,2006). Findings of Stein *et al.,* (2006) showed that pigs fed a P-free diet were able to absorb calcium in the intestine, because there was not enough phosphorus (P) to retain the absorbed calcium in the bones, the absorbed calcium wass excreted in the urine.

2.9.13 Calcium bioavailability

Bioavailability is the term used in the measurement of Ca availability in chickens fed the inorganic form of calcium. In some experiments the digestibility of calcium is represented by a graph where the birds on a Ca deficient basal diet, and those on the test diets at different inclusion levels. It is always within 2-3 weeks of introducing the experimental diet and the different parameters like bone strength, bone ash, weight gain, bone calcium content and serum calcium concentration are compared with the groups fed diets with inorganic Ca source (Mclean and Urist, 1961). That is the relative bioavailability of the quantity of Ca absorbed from the experimental diet. Walk *et al*. (2012) reported a reasonable range 28% and 66% apparent ileal Ca absorption in a corn-soybean broiler starter diets. No nutrient is totally digestible and Ca is no exception because it is assumed to be highly available.

2.9.14 Calcium deficiency

Inadequate calcium can cause bone deformity and poor calcium absorption that reduces bone mineralisation, for example osteoporosis and rickets that are caused by deficiency of Vitamin D. It inhibits the intestinal absorption of Ca which results into decrese rate of bone mineralization (Burrell *et al.,* 2004). The bones develop into soft, pale and deformed. However, some research suggests increased risk of some diseases

is associated with high intake, especially from supplementation of calcium but the information concerning varying inclusion of calcium and oil has not been fully investigated (Soares, 1995). It has been reported that with high calcium intake, low body weight and body fat is obtained (Liu and Stein 2014), and a decreased appetite, fat absorption and increased lipolysis and thermogenesis is proposed as a means to describe the effect of excessive calcium intake (Murphy, 2009).

The effect of high dietary Ca intake could be seen in blood and the serum lipid profileas a result of digestion of the oil and Ca in the GIT and high Ca intake could also lead to high faecal excretion of bile which leads to a high demand for cholesterol meant for the renewal of bile acids thereby reducing hepatic cholesterol. A slight drop in the hepatic cholesterol increases the LDL receptor and increase the uptake of LDLfrom the body thereby lowering serum cholesterol concentrations. An increased Ca intake could reduce cholesterol absorption. In some invitro studies, it has been reported that high Ca intake coud hinder intestinal cholesterol transporters and ATP thereby reducing cholesterol absorption (Seyrek *et al*., 2004). Calcium forms insoluble soaps in the GIT and reduces the absorption of some fatty acids and SFA are not utilised by Ca compared to PUFA or MUFA (Atteh and Leeson, 1983).

2.10 Absorption of calcium in birds

Absorbed calcium in the GIT contributes to birds metabolic needs. Calcium in natural feedstuff from plant most times are present as phytate and oxalate complexes, thereby reducing its availability. Normally, a corn-soya beans meal diet contains less than 1% calcium without supplementation. Calcium is important in poultry birds through their involvement in supplying structural vigor for avian bones with a crucial function in biochemical reaction processes (Kim and Wyckoff, 1990). Calcium could be analysed in three different forms in serum of avians; an ionised salts, calcium inherent in some anions. The physiological portion of serum calcium is the ionised calcium useful in bone homeostasis, muscle and nerve condition, blood coagulation, hormone emission especially cholecalciferol and parathyroid hormone.

Indigenous chickens will revise hypocalcaemia difficulties compared to challenged mammalian response over a period of 24hours. Laying bird’s requirement is about 10% addition for egg production as their dietary calcium intake. The calcium needed

by pullets is obtained by an high intestinal absorption and extremely labile pool located in the medullary bone. Laying chickens fed a diet deficient of calcium will stop laying when plasma calcium concentration is reduced. Bone defects such as rickets, osteomalacia, osteoporosis, tibia dyschondroplasia, and hypocalcaemia partially ascribed to reduced digestion and inadequate calcium concentration have led to unprofitable yield in the poultry business. The cost-effective impact of Ca in poultry has enhanced the unreserved investigation into calcium absorption. Once inorganic supplemental calcium is included into the diets of poultry birds, additional calcium inhibits absorption of other minerals which include phosphorus, magnesium, manganeses and zinc (NRC, 1994).

Burrel *et al.*, (2004) reported that calcium found in egg shell are derived mainly (60-75%) from dietary source and from medullar bone. Laying birds with a reduced plasma calcium concentration of 1.0mmol/L will stop laying due to insufficient Ca in the diet. Major Ca content is located in the skeleton while others found their importance in cellular digestion and blood clotting. Ionic calcium present in blood plasma is preserved by guiding process that regulates calcium homeostatic hormones. Calcium need is different at every stage all through the life of an animal. The body weight of chicks increases rapidly from day one to about three weeks old, this was observed when a concentration of about 350mg calcium/kg of of calcium was added to the diet (Soares, 1995).

Soares, (1995) fed varying levels of dietary calcium at a constant phosphorus concentration to weaned pigs and observed that increasing levels of calcium resulted in faecal excretion of phosphorus, but urinary P output reduced. He also stated that the digestibility of calcium along the total tract was not influenced by increasing dietary absorption of calcium through lowered digestibility of phosphorus. Liu *et al*. (2000) fed growing pigs with an increasing level of calcium via calcium salt at the same level with phosphorus and reported that calcium absorption was not influenced while total tract phosphorus digestibility decreased. The authors asserted that greatest value for apparent phosphorus digestibility is acquired if the nutritional Ca: P is slightly less than 1.1:1. Supporting these findings is the research work of Murphy, (2009) who observe that serum phosphorus concentration, tibia ash and bone breaking strength

reduced as the Ca: P raised from 1.3:1 to 2.0:1, 3.0:1. Therefore, the opposing effect of Ca and P depends on their quantity in the feeds.

Calcium absorption through the intestinal wall occurs through two pathways known as trans and paracellular pathways. However Shafey and McDonald (1991) observed that transcellular effective transport occurs mostly at the upper segment of the small intestine, particularly in the upper jejunum and duodenum in numerous animals. Transcellular calcium absorption follows the epithelial calcium routes from the intestine to the enterocyte. Intracellular calbindin for trans-cystosolic circulation and ATP triggered basolateral membrane calcium pump. Vitamin D_3 involvements in each of these steps is compulsory for calcium absorption. Recently, it was reported that calcium absorption in the ileum occurs principally via vitamin D_3- autonomous paracellular pathway and concentration determined (McCormick, 2002). As reviewed by Adedokun and Adeola, (2013) *in vivo* and *in vitro* studies did not oppose the wealth of the individual carriers of calcium in the higher section of the intestine. The jejunum and ileum length as well as the time the digesta stays in the jejunum and ileum offers an alternative cause while paracellular absorption of calcium and phosphorus might donate meaningfully to the quantity of these minerals absorbed. The pathway in the small intestine take in calcium in three different ways. First calcium in the lumen arrives in the enterocyte by a network of amino acids that are positioned in the film while transcellular absorbption of occurs in the lumen and arrives in the enterocyte by a network of proteins to the needed cells, (Taylor and Buskinsky, 2009)

2.10.1 Synergistic effects of calcium and phosphorus in broilers

The two major minerals involved in animal diets are calcium and phosphorus. It has been documented that the two minerals have a synergistic effect on the predominant impact of mineral balancing. Among relationships existing between minerals, the most investigated contact amidst minerals exist between these two minerals. Calcium and phosphorus are intently linked together due to their use and their close storage structure (Lima *et al*., 1997). Concentration of phosphorus is often calculated either as total phosphorus, available phosphorus and calcium to total phosphorus ratio (NRC, 1994) in formulating diets when a grain-soybean meal based diet is being fed to chickens, the Ca and P should be amidst 1.1:1 and 1.25:1 (NRC, 1994). However,

when available phosphorus is used in formulating the diets, calcium and available phosphorus will be in between 2:1 and 3:1 (Quian *et al*., 1996).

2.11 Quality control in oils

Vegetable and animal oils are the main source of dietary lipids. Corn oil, cottonseed, Palm oil, sunflower, olive, soybean, coconut, and groundnut oils are some of the oils of vegetable origin widely used. The domestic use of fish oils has not been known world wide due to lack of a good technological know how in the production of fish oil without the loss of n-3 PUFA and several important components. The liquid content can be ascertained through the size of the lipidic source in percentage. Acceptable values ranges between 0.5 and 1.0% for the optimum values, because the rate of moisture has great effect on the energy content of an oil (Butolo, 2002).

Adulterations are obtained as the fraction, insoluble in petroleum ether at higher temperatures of 40-60^{o}C and results obtained in percentage. Contents termed impure should not be up to 1%. Acidity is the amount of free fatty acids from NaOH titration with oleic acid predominant in saturated fats with results obtained and written as mg of NaOH/g of fat. Fatty acids are determined by methyl esters separation using gas chromatography. The fatty acid outline determines the quality of lipids and the extent of its utilisation by birds. Oxidative rancidity occurs due to the oxidation of double bonds in unsaturated fatty acids forming peroxides that latter polymerise and decompose with the production of aldehydes, ketones and low molecular weight acids. The process of oxidative rancidity also known as peroxidation and the consequent rancidification is the major cause of loss of quality if the ingredient in the diet formulation. It affects the flavor, aroma, color, texture and decreases the nutritive value. Also fat-soluble vitamins are destroyed in the process, especially vitamins A and E (Shermer, 1990). Oils and fats that composed mainly of by unsaturated fatty acids sufer the attack of free radicals. The chemical structure allows the removal an hydrogen atom from a CH_2 group of the carbonic acid and consequently a free radical is formed, starting a lipidic peroxidation process. This reaction may occur at environmental temperatures, but can be rapidly increased in the presence of catalysers such as other peroxides, copper, iron, nickel, cadmium, zinc, high temperatures and light. The combination of several free radicals of fatty acids produces a huge variety of stable final products hydrocarbons, aldehydes, ketones, alcohols and organic acids

(Menten *et al*., 2003). Hydrolytic rancidification results in the development of undesirable flavor due to the hydrolysis of the triglycerides that compose fats and oils as a result of the action lipolytic enzymes present in the fats or produced by some microorganisms.

Peroxide value: is a method of testing the stability and purity of oils and fat. They are compounds with high rate of instability and their concentration has a characteristic normal curve. This kind of test is carried out periodically because there is a decrease in peroxide formation while the decomposition of secondary product lasts (Shermer, 1990).

Iodine value: is a measure of fat stability. It measures or evaluates double bonds inherent in the oil, as this is important. Thiobarbituric acid (TBARS) evaluates the quantity of malonaldehyde obtained from triglyceride oxidation. In peroxidation reaction, malonaldehyde is a product formed initially from the triglycerides oxidation. However, this method is not without its limitation because before malonaldehyde is formed, oxidation process could occur, although malonaldehyde is the only product derived from the composition of peroxides among several others.

Saponification value: This is the alkaline hydrolysis of oil. It measures the volume of an alkaline needed to dissolve the acid present in a sample of oil. The carbon chain length has effect on the saponification values (Butolo, 2002).

Fatty acid profile: It is determined by measuring the composition of the methyl esters of the fatty acids through several means e.g gas chromatography. This is done because the composition has effect on the final product after the feeding trial (Barbi and Lúcio, 2003).

2.12 Dietary oil types available to poultry birds

2.12.1 Cotton oil

Gossypol is the only anti-nutritional element present in cotton oil that inhibits its use as a feed component. It is yellow in colour. It inhibits availability of iron and some essential amino acid reactions and reduces dietary evaluation of proteins when incorporated in the diet. If cotton oil is added to the diet of broiler chickens, ferrous sulphate must be included because of it's chelating action that prevents absorption

along the digestive tract thereby counteracting its harmful effect. Broiler chickens can accept gossypol contents above 100 ppm with no noticeable damage. When gossypol is ingested it increased quantity, the yolk develops a colour change after a short period of refrigeration. The change in colour could be associated with yolk's increased content of saturated fatty acids, that stops stearic and palmitic acid denaturation alongside other mono unsaturated fatty acids. Pink pigmentation in the albumen is a product response between ovotransferrin and iron. Also, a part of the ovotransferrin migrates to the yolk, mix up with iron resulting in red colouration. However, in breeding birds increased gossypol, yolk staining and ovotransferin transport may reduce hatchability of eggs (Butolo, 2002)

2.12.2 Palm oil

Palm oil is a product of the oil palm tree, capable of growing to about 30meters; producing fruits which could be spherical in shape, big, compact and could make up to 2,000 fruits each. These tropical fruits are source of volumes of oil about (45-65%) that are reddish in colour due to high carotene content. The kernel of the fruit is used in producing another oil different from palm oil called palm kernel oil. Palm oil is obtained through pressing of the pulp of the fruit after heating. The heating process is applied to purify the crude palm oil, providing oil with diverse chemical properties. It can also be separated into fats and oils and processed according to the purposes it is meant for, to give taste in different food products.

Palm oil is made up of saturated fatty acids and unsaturated fatty acids (50:50). To obtain the same creaminess with palm oil, other vegetable oils with low SFA contents need to undergo hydrogenation in the final product and hydrogenation leads to transfatty acid which is nutritionally unacceptable. Palm oil has been in use in different scientific research studies demonstrating the neutrality of the effect of palm oil on metabolism as a component of a balanced diet. Ekpa, (1995) reported that palm oil, FA obtained from the palm are rich sources of vegetable oils with a fatty acid profile which could substitute for animal fats with no adverse influence on carcass quality.
In a study where different vegetable oils were compared to palm oil: Olive oil (a MUFA and an Oleic), sunflower oil (PUFA and Oleic acid), soybean oil(more PUFA and less SFA,) and canola (a MUFA), was compared with soybean oil (a vegetable oil

with more PUFAs and less SFAs), with olive oil (rich in oleic acid, a MUFA), with sunflower oil (rich in oleic acid and PUFA) and with canola oil (rich in MUFAs), Fattore and Fanelli, (2013) reported that there were no differences in the serum lipid profile. Unrefined palm oil is made up of about 100mg of vitamin E in 100g of oil while a refined palm oil contains an average of 50- 65% of the vitamin E and they occur as tocotrienols.

Carotenoids act as precursors of vitamin A, and it plays a crucial role in good vision, a healthy immune system and good growth. The pro vitamin A (retinol) equivalent content of crude palm oil has been estimated at 15 times that of carrots.

2.12.3 Acidulated soybean oil soapstock

The fatty acids of soyabeans is referred to as soya bean oil soapstock, obtained through alkaline nullification of the unrefined oil to give soaps containing neutral oil, water, sterols and pigments. However, it is volatile and so converted to acidulated soyabeans oil soap stock when treated with sulfuric acid. Acidulated soyabeans oil is made up of free fatty acids in large quantities, unsaponifiable matter, carotenoids and oxidised fatty acids compared to soya beans oil (Pardio, 2001). The understanding of the quantity of free FA plus the inherent triglycerides is crucial because additional acids are less utilise compared with the free fatty acids in triglycerides forms. Monoglycerides are crucial in the utilisation of insoluble fatty acids in the micellar complex. When fatty acids are included in the diets as the only fat source, there will not be an adequate amount of monoglycerides to couple with all free fatty acids, thereby absorption is greatly reduced.

Wiseman and Salvador (1991) performed an experiment with broilers chickens and added different sources of oils into their diets (tallow, soybean oil and palm oil). As the oil inclusion increases, there is a decrease in metabolisable calorie values of the fats which is more prominent with higher inclusion levels of lipids.

2.12.4 Beef tallow

The fat type included in diets of broiler chickens play predominant function in determining the constituent of fatty acid in the carcass of broilers. In a study by Newman *et al*. (2002) where broilers were fed with diets containing 8% of beef tallow or sunflower oil and observed a poor feed efficiency in the birds fed diet containing

beef tallow. There was an increased essential fatty acid composition in the thigh, breast and skin of broilers fed diets containing beef tallow or corn oil or the inclusion of both (Sanz, 1999). Increased quantity of abdominal fat was recorded for broilers fed diet containing high level of saturated fatty acid than broilers fed diet with soybean oil inclusion (Thacker and Campbell 1994).

Also in another study involving the use of sunflower and beef tallow as energy source and included at 8%. Sanz, (2000) reported a reduced fat deposit in the abdominal fat in birds that were fed diets with sunflower oil. Crespo and Esteve-Garcia (2002), reported that the point where the fat is located is a determinant of the type of FA incorporated into the diet (SFA and PUFA). Birds fed with diets rich saturated fatty acids from animal source tend to have comparably larger abdominal and mesenteric fat than other fat deposits.

2.12.5 Fish oil

Fish oil is obtained through hydrolic squeezing of fishes and other products for fish production. The products obtained are made up of high-level of PUFA that is responsible for the unstable flavor of fish and distinctive taste to the meat of birds fed this oil. However, fish oils are good sources of omega-3-fatty acids. The type of fish used in the production of fish oil and the season, has a great effect on profile of oils (Dvorin *et al.,* 1998). Newman *et al.* (2002) reported 8% inclusion of dietary oils from sunflower, fish or beef tallow in broiler rations as uneconomical and also has led to reduced abdominal fat deposition on fish and sunflower oils.

Phetteplace and Watkins (1990) evaluated different fractions of poultry fat and fish oil in broiler feeding and reported that broilers fed with diets containing more fish oil have deposited more quantities of unsaturated fatty acids in the abdominal fat, as well as more n-3 fatty acids. On the other hand, bothe total n-6 fatty acids and the relation n-6:n-3 were higher in the abdominal fat of birds fed with rations containing higher quantities of poultry fat. However, Chanmugam *et al*. (1992) also demonstrated that the content of w-3 fatty acids in the thigh of broilers might be increased by the addition of linseed oil or fish oil in the diet. Diet inclusion of 8% of sunflower oil, fish oil or beef tallow resulted in lower deposition of corporal fat on birds fed with fish oil and

sunflower oil. It was concluded that feeding broilers with sources of n-3 and n-6 produced less carcass fat amd improved feed conversion ratio.

2.12.6 Coconut oil

Coconut oil was described as light yellow oil of slightly low melting point that ranges between 23° to 26°c (Gopala Krishna *et al*., 2010). Coconut oil contains the highest quantity of glycerol of about 13.5% to 15.0%, it is a colourless liquid with a pleasant aroma and a composition comparable to that of simple sugar and glycerol this makes it a good source of energy, the fatty acid intake is less than what is obtained from coconut oil. The quantity of SFA present in coconut oil exhibits properties that are specific to short and MCFA that can digest easily and utilized via the portal vein to generate energy (Huiling and Carl-Erik, 2004). MCFA are characteristics of fatty acids of 12 carbons or less. Coconut oil has a stable chemical constituent irrespective of location of origin.

Coconut oil is obtained from the milk of fresh, ripe kernel of coconut *Cococ nucifera L.,* which is a tropical plant and useful in cooking, costmetics, confectionary and pharmaceutical uses. It can be obtained through mechanical and physical means which does not require any further processing either by refining, bleaching or deodorization. Huiling and Carl-Erik, (2004) reported that coconut oil, cuphea oil, cohune oil, babassu oil are the oil with medium chain fatty acids in high amounts (C8:0- C12:0). MCFA has some unique nutritional properties which include antiprotozoal, antibacterial, antiplaque and antiviral effects (Gopala Krishna *et al*., 2010). These properties enhances it's multipurpose use. MCFA has been recognized as multipurpose nutritional supplement because of it's medicinal benefits. However, the quality of the coconut oil is based on the quality and breed of the copra used in the processing and the techniques involved in the coconut oil production.

Coconut oil is good nutritionally for patients suffering from digestive illnesses particularly in absorbing fats. In men, coconut oil is practically fully digested compared with other nutritional oils. It is also an important constituent of newborn diet formula in the management of undernourishment. Mineral uptake especially, calcium and magnesium as well as amino acid have been known to be on the high side when newborns are given diets containing the inclusion of coconut oil whenever there is

deficiency of nutrients. For the elderly, it is important in reducing the generative process by aiding mineral uptake. Moreover, it facilitates the supply of energy to cells due to its ease of digestion and absorption of nutrients, helps insulin irregularity and use of glucose in the blood (Taylor and Bushinsky, 2009). Animal's immune system is greatly affected by polyunsaturated oils; they create a shift in the immune system into a feverish activity also affecting protective compounds formation. Free radical scavengers whose activities are pronounced when unsaturated oils are included in the diets are known as antioxidants. Examples of antioxidants include Vitamin A, C and E. When these become exhausted, the rate of defence of the immune system is reduced.

Coconut oil has been proven to have a profound effect in protecting the body system against harmful pathogens compared to other vegetable oils. Coconut oil is non-poisonous and does not put excess load on the immune system. On the other hand, saturated fats are very stable and do form micelles readily, thereby preventing the inherent antioxidants from their scavenging activities. Coconut oil contains about 63% antimicrobial MCFA, and very effective in protecting the cells from invaders. Coconut oil contains all the constituents that make up a triglyceride molecule and it's microbial properties which includes: lauric 48%, capric acid 7%, caprylic acid 8% and 5% caproic acid. Mammary gland contains MCFA which serves as a defence for the new born from dangerous microbes. MCFA has been an important constituent of infant formula for years, due to its ease of digestibility and absorption of nutrients. Nursing mothers are encouraged to take more of coconut oil because of it's MCFA nature that help protect and nourish the newborn baby, because infants obtain their lauric acid from their mother's milk, because it has been demonstrated through several laboratory research to be efficient in annihilating viruses that could result into influenz*a. Monoglyceride monolaurin* is a source of protection for infants against viral or protozoa or bacterial infections (Gopala Krishna *et al*., 2010).

2.12.7 Groundnut oil

Groundnut is an oil seed crop known for it's protein rich kernel seeds enclosed in a pod as well as it's edibility. It is an annual crop cultivated all over the world which matures inside the soil. About 44-56% oil could be obtained from the seed and minerals such as Mg, Ca, P and K. Groundnut oil has a vital influence in diets among

several tribes; being a cheap supply of protein, oil and fatty acids for animal and human nutrition. The monounsaturated quality and characteristic flavour makes it generally acceptable oil (Huiling and Carl-Erik, 2004). Groundnut oil is also rich in antioxidant α-tocopherol. Vitamin E is a potent antioxidant, needed for sustaining the integrity of cell membrane and skin from destructive free radicals.

2.12.8 Palm kernel oil

Palm trees are known for bearing bunches of fruit that weighs between 40-50kg. The drupe, pericarp and seeds (kernels) are useful in soap and vegetable oil production. Oils obtained from the pericarp are processed and used in cooking (Ekpa, 1995).

Palm kernel is rich in both organic and inorganic minerals especially potassium and calcium (Ekpa, 1995). Palm kernel oil and palm oil and are some of the products obtained from the fruit of the palm tree and they contains different chemical constituents and use. The chaff obtained from the extraction of the oil is included in livestock feed, while the kernel is one of the component of soap making. Palm Kernel oil is second to coconut oil in its fatty acid composition, containing high level of lauric (Berger *et al*., 1991). Apart from their industrial use, they are used as cooking oils, antidotes for poisoning, body creams and in the treatment of wounds. Some oils like canola and olive oil s are rich in Oleic acid, while sunflower, soybean and cottonseed oil has high content of linoleic acid while groundnut oil is rich in both fatty acids.

It contains sufficient quantities of α-tocopherol, rarely found tocotrienol which serves as antioxidants providing amazing anti-aging benefits. The palm kernel oil could be grouped as unsaturated oil as well as a medium chain fatty acid. Also, this inherent property enables healthy bones and joints movement. The fat-soluble vitamins present serves as a good blood coagulant, corrects visual impairment like night blindness

2.12.9 Shea butter oil

Shea butter (*Vitellaria paradoxa*) is obtained from the kernels of shea nut from matured shea-trees, contains high level of saturated fatty acid which makes it to be semi-solid at room temperature. Shea butter is obtained from its nuts and widely used in cooking as one of the components, while the pulp of the ripe fruit is used as feed for small ruminants. Dennie, (2012) reported that the oil obtained from extraction is a

source of relieve in headaches and sores.The FA contents vary with location and it is one of the ingredients used in making cosmetics and chocolate.

Reports from FAO (1988) stated that the leaves of shear butter are used as part of the component in treating aches in children and adults. Chemically, it contains Iron 1.90-100g, protein 0.7-1.3g, carbohydrate 41.3g -100g, calcium 34.4mg -100g and ascorbic acid 196.1mg -100g respectively. Shea butter contains vitamins A and E, as well as catechins, plant antioxidants also found in green tea (Chew, 1995). It has several health benefits such as its useage in the treatment of rheumatism, inflammation, cough, catarrh and slight dislocation (Dennie, 2012). Also used in infants as a moisturizer for smooth skin and to clear unwanted spots due to its antibacterial properties. The presence of Lupeol which is a pentacyclic triterpenoid with anti oxidative properties helps in preventing swelling caused by excess water in the body tissues (edema). Shea butter could be easily obtained from an open market at a relatively justifiable price, this makes it acceptable as a cooking oil. It serves as a lubricator in lamps, heating oils as well as a base in soap making.

The saponifiable component of shea butter is about 90% and this accounts for the emollient properties used in body creams. The unsaponifiable fractions (hydrocarbons, tocopherols, sterols) contributes the active ingredients in its medicinal functions (Esuoso *et al.*, 2000). The unsaponifiable fraction consists of triterpene alcohols, hydrocarbons sterols, alpa tocopherol which is most abundant of all the isomers of tocopherols inherent in the oil (α, β, λ).

2.12.10 Soya bean oil

Soya bean oil (*Glycine max*) is generally acceptable cooking oil, obtained from the seeds of soya beans which is listed among legumes. Soya bean oil is obtained from heating the cracked beans to a temperature between 60 and 88°C, grinded and cut into small portions and the oil is squeezed out by hydrolic pressing. The extracted oil is subjected to hydrogenation and refined for several purposes and the chaff is used in animal feeds. Soya bean oil is categorized as a polyunsaturated fat due to the essential fatty acids present in the oil. α-linolenic acid C-18:3, linoleic acid C-18:2 and oleic acid C-18:1 and a less quantities of stearic acid C-18:0, palmitic C-16:0. These qualities accounts for its suitability in frying, baking as well as an ingredient in salads.

It also aids bone resorption due to its inherent fat-soluble vitamins that is associated with mineral absorption especially calcium and vitamin K in the oil that helps blood coagulation. Lara *et al*. (2003) fed male broilers with different lipidic sources (degummed soybean oil, poultry fat, acidulated soybean soapstock, and a mixture of poultry fat). He however reported better weight gain and good feed intake in birds fed soybean oil compared to birds fed acidulated soybean oil soapstock. The different oil sources had no influence on the levels of moisture, ether extract, and protein of the breast, thigh and whole carcasses. While Moura (2003), reported that the inclusion of soybean oil in broiler diets does not affect the moisture and ether extract in the breast and thigh muscles.

2.13 Carcass traits

The end product of broiler chicken production is to be able to meet up with the rapid demand for poultry meat. The quality and quantity are more determined by environmental factors and genetics. A large portion of the quality of the meat is geared towards lean meat production which is related to the diet fed to the chickens (Waldenstedt, 2006). In a study on different dietary oil sources, Ca and P levels on carcass weight, dressing percentage, leg and breast cut by Abdulla *et al*., (2017), he observed no significant differences among the chicken fed different dietary oils in their dressing percentage, breast percentage and total weight. The economic cut of the carcass portion were not influenced by the oil sources Zollitsch *et al*. (1997) reported similar results when a blend of soybean oil, rapeseed oil and processed oil product was included in the diet of broilers.

Also, Lopez-Ferrer (1999) observed no significant difference in the thigh, breast, dressing percentage and drumstick muscle contentsthere was no significant difference when broilers were fed diets supplemented with linseed oil, soybean oil and rapeseed.. However, El-Yamany *et al*. (2008) stated that there were significant differences in carcass weight of Japanese quail fed diets supplemented with sunflower oils, linseed and fish oils, but there was no influence on different varying Ca levels on leg, breast and carcass yield.

Also carcass weight was reduced in broilers fed diets supplemented with 1.5% calcium compared to other inclusion levels, this corroborates the findings of Rodehutscord (2005) who stated that there was no influence of Ca on the dressing percentage of

broilers when varying levels of Ca was fed which was contrary to the observation of Abdulla *et al*., (2017) that an increased dressing percentage was obtained when broiler chickens were fed diet containing 10g/kg of Ca.

2.14 Bone characteristics

Bone criteria has been a reliable tool used for determining Ca availability in poultry, as bone mineral uptake is a vigorous procedure involving adaptations and adjustments to physiological changes. Parameters of interest usually include bone ash, phosphorus concentration, and bone breaking strength. Left tibia bone is mostly selected for the examination of phosphorus and calcium alteration. Shastak *et al.,* (2012) also noted that the ash obtained from tibia, femur, and toe ash has also been used as a response criterion for calcium and phosphorus availability studies. Bone breaking strength and bone mineral density were used as rapid indicator for mineralisation. Lameness, fracture and some other bone problems are some of the problems facing poultry production due to their rapid growth rate. This usually results in great to loss to the farmer and raising the cost incurred in raising a bird to market weight (Waldenstedt, 2006). In a study by Abdulla *et al*. (2017) on the effect of dietary oils on calcium and Phosphorus levels on bone characteristics, it was observed that birds fed diets supplemented with linseed oil had higher bone breaking strength, tibia ash, tibia weight than birds with diet supplemented with palm oil and soybean oil. According to Atteh and Leeson, (1983), they reported a significant reduction in tibia ash, tibia calcium concentration of chickens fed diets with palmitic and oleic acid inclusion in their diets. He attributed this to complete digestion and absorption of unsaturated fatty acids (oleic) due to soluble soap formation as opposed to insoluble calcium soap formation with saturated fatty acids (palmitic acid) in pullets and broilers. Also, Shafey and McDonald, (1991) reported a consistent and beneficial effects of omega-3 fatty acids on bone metabolism which led to a healthy bone growth. However, Murphy, (2009) observed a decreased bone thickness and high fragility in monogastrics. Also, there was a notable increase in the thickness of the lateral wall, tibio tarsal index, bone breaking strength, tibia ash of broilers fed diets with 1.25% Ca inclusion than birds fed 1.00% Ca. Shastak *et al*. (2012) observed high tibia ash with increasing level of Ca and phosphorus up to 5.0 g/kg phosphorus and this agrees with the reports of Jongbloed and Kemme, (2002) that an increased dietary Ca and P in broilers diet increased the

content of tibia ash but bone breaking strength was not affected.The genetic ability of poultry birds tends to set a limit for Ca and P digestibility. However, this limit is not fixed and changes with advances in genetics and breeding, as well as the full genetic potential of the bird.

2.15 Physiological parameters

Physiological parameters are some of the criteria used in evaluating the effect of feeds on some vital organs in the life of an animal e.g. serum cholesterol levels, triglyceride levels, aspartate amino transferase, alkaline phosphatase.

The strength of using physiological measures as an indicator of competence is that they are quantifiable procedures that can quantify disease risk before experimental trials. The only defect is that these parameters are not directly linked to the actual cause of a disease in studied. They are limited to a particular pathway of symptoms observed and may not reveal the root cause of the disease in relation to the type of the test ingredient fed to the animal.

2.15.1 Superoxide Dismutase (SOD)

Oxidative stress is one of the problems facing poultry production in the tropics. It occurs when the immune defence of the body could no longer detoxify the reactive species produced by reactive oxygen species. Extremely high levels of oxidative stress causes a lot of damage to lipids, proteins and DNA which reduces performance, increased mortality, poor meat quality and eventually cell death (Davies *et al*., 1970). Also, phagocyte cells produce oxygen radicals in an attempt to destroy invading harmful microbes. These products may also result in destruction of cells that are healthy if not quickly mopped. The role of antioxidants becomes eminent to stabilize the reactive oxygen species in order to maintain the functionality and the cell integrity (Chew, 1995). One of the methods in estimating oxidative stress in animals is to measure some nucleic acids, proteins, oxidised lipids and from blood samples.

The antioxidant defence system plays a key role in the reduction of the heat stress generated lipid peroxidation process (Lin *et al*., 2006). In the acute phase (heat stress), the antioxidant-prooxidant balance is upset, with the balance shifted to the prooxidant phase. To re-establish this balance, the antioxidant defence system will be activated.

The elimination of the free radicals activates the three level antioxidant system (Min *et al*., 2018).

This first level (direct enzymatic pathway) includes the neutralization of free radicals by enzymes. The principal enzymes which regulate this are superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) (Jeeva *et al*., 2015). The second level includes the detoxification and regeneration reactions of the small molecule antioxidants working at the same time with the first level: Vitamin A, E, C and glutathione (Irshad and Chaudhuri, 2002). The third level is activated when damaged systems (proteins, DNA) have to be repaired and/or removed from the cells by chaperones and DNA-repair enzymes (Irshad and Chaudhuri, 2002).

Poultry is more sensitive to heat stress than other domestic animals, because they do not have sweat glands, their metabolism is rapid and they have high body temperatures. Increased environmental temperature causes increased lipid peroxidation induced the formation of malondialdehyde (MDA), which is an indicator for lipid peroxidation). Therefore, the antioxidant defence system is altered (Altan *et al*., 2003; Chauhan *et al.,* 2014; Costantini *et al*., 2009; Lara and Rostagno, 2013).

The superoxide dismutase is one of the serum enzymes that has the first line of defense by converting harmful free radicals to hydrogen peroxide that is less harmful to the body system and finally to water. In an animal cell, there are different types of SOD: the zinc and copper dependent form in the cytoplasm and the manganese-dependent form in the mitochondria (Liu *et al.,* 2000). Hsu and Guo, (2002) reported that in a mineral deficient diet SOD activity is reduced which could lead to an increased amount of lipid, nucleic acid and protein damage which may lead to death.

2.15.2 Triglycerides

Triglycerides (TG) are simple lipids made up of fatty acids and glycerol, which are major storage form of fat and are primarily derived from fats manufactured from excessive caloric intake or from the consumed fats. The majority of fat tissue is made up of triglyceride. A high triglycerides level is often accompanied with higher LDL, high total cholesterol, levels and a lower HDL cholesterol levels (Sacks and Katan,

2002). Digestion of triglycerides requires bile acids which has an influence in the digestion of fat and produced from cholesterol (Hemme *et al*, 2005).

2.15.3 Alkaline phosphatase

The evaluation of blood parameters, enzyme activity and the oxidative status of some organs, allow a precise estimation of the health and nutritional status of the birds, together clarifying the effects of the test ingredient in poultry which leads to evaluation of the effects on blood parameters and the enzymatic and oxidative activity in the liver (Özek, 2011) Alkaline phosphatase (ALP) is a biochemical catalyst that is capable of eliminating phosphate groups from several type of particules like nucleotides and proteins, in a process known as phosphorylation (Nourmohammadi, 2010). The main site of production is in the liver and bones, it is generated from the intestine, kidney and placenta, liver, gall bladder malfunctioning are associated with increased level of serum ALP responsible for obstruction of the biliary duct leading from the gall bladder through the pancrease and into the small intestine. Rapid bone growth and the efficiency of vitamin D in young chicks could also lead to an increase in serum ALP, because bone forming cells produce ALP. That is why higher ALP levels are recorded in young poults than matured ones (Hemme *et al*., 2005).

2.15.4 Aspartate aminotransferase

Aspartate Aminotransferase (AST) and Alkaline phosphatase (ALT) activities of the serum may indicate the liver function and health of the hosts.

Cellular injury in liver may increase the level of these enzymes in serum. ALT was principally found in the liver and was regarded as being more specific than AST for detecting liver cell damage. The increased WBC count could be attributed to improvement in the immune system of the chickens brought about by improved stimulation by inducement by probiotics. This stimulates a strong immune response and induces a type of antigen-antibody reaction that was responsible for the clinical signs. At the same time, the possible presence of the bacterium in target organs such as; the liver, spleen, kidneys, thymus and heart, may stimulate the production and release of leukocytes into the blood stream (Abdel-Fattah 2008)

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CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site

The experiment was carried out at the Agricultural Biochemistry and Nutrition Laboratory, Department of Animal Science and the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. It is situated at the derived savanna vegetation belt on (latitude 7°27'N and 3°45'E) at an altitude between 200m and 300m above sea level. Mean temperature 25-29°c with an average annual rainfall of about 1250mm. The soils are much drained and belong to the alfisol (Rhodic Kandiustalf) Babayemi *et al*., (2013).

3.2 Study one: Physico-chemical attributes of selected dietary oils

Coconut oil, palm kernel oil, shea butter, groundnut oil and soya bean oil were purposively selected among others and purchased from an open market and thereafter subjected to physico-chemical analyses. The oils were selected based on their degrees of unsaturation which aids digestion and absorption of nutrients, length of the carbonic chain which enhances higher lipase activity and micelle formation within the gastrointestinal tract, α-tocopherol and total carotene contents which are natural antioxidant that protects the cell membranes against lipid oxidation. The saponification, peroxide, acid, iodine values, fatty acid profile, α-tocopherol and total carotene contents were determined according to AOAC (2000).

3.2.1 Saponification Value (SV)

2 mL of each oil sample was carefully poured into a conical flask and 0.5N ethanolic potassium hydroxide was added and refluxed for 45 minutes with occasional shaking

till it become colourless. The solution was left to cool afterwards and was titrated with 0.5N sulphuric acid with phenolphthalein as indicator. Blank sample was also prepared (AOAC 2000; method no: 920.160)

SV was calculated with the formula $= \frac{(Vb-Vs) \times 28.05}{W}$ ($mgKOHg^{-1}$)

Vb = value of blank: Vs= value of sample: W= sample weight (g)

3.2.2 Iodine Value (IV)

2 mL of oil sample was dissolved in chloroform and titrated against 5 mL Wij's (iodine monochloride) solution and mixed evenly. The solution was then sealed with a black polythene and kept in a cupboard to avoid ray of light for five minutes. 5 mL of 75% potassium iodide solution was added and then titrated to a light straw colour with 0.1N sodium thiosulphate using three drops of starch indicator (AOAC, 2000; method no: 968.08).

IV was calculated as $= \frac{(Vb - Vs) \times 1.269}{W}$ (as $I_2 100g^{-1}$)

Vb =value of blank; Vs = value of sample, W= sample (g)

3.2.3 Peroxide Value (PV)

2 mL of the respective oil sample was poured into a glass tube containing 1g of potassium iodide and glacial acetic acid (20 mL) was added into the test tube and boiled for 1minute in a water bath. The tube was left to cool and the contents emptied into a conical flask containing 20 mL of 5% potassium iodide solution and then titrated against (0.002M) sodium thiosulphate using starch as an indicator (AOAC, 2000; method no: 965.33).

PV was calculated with the formula

$= \frac{(Vs - Vb) \times \text{molarity of titrant} \times 103gkg^{-1}}{W}$ ($mMol/kg^{-1}$)

Vb = value of blank: Vs= value of sample (mL) W= sample (g)`

3.2.4 Acid Value (AV)

Approximately 2 mL of the oil sample was poured into a conical flask containing a mixture of ether 50 mL and 95% ethanol. The solution was titrated with potassium hydroxide solution using phenolphthalein as an indicator. Results obtained were expressed as $KOHg^{-1}$ (AOAC, 2000; method no: 660.1996).

$$\text{AV was calculated with the formula} = \frac{(Vb - Vs) \times 5.61}{W}$$

Vb = value of blank, Vs = value of sample; W= Sample (g).

3.2.5 Fatty acid profile

100mg of oil was dissolved with 1.2 mL of 0.5M methanolic KOH at 60°C for 10minutes, with 0.7M HCL and methylated with 3.0 mL boron trifluoride methanol complex solution (BF3-CH3OH) in a water bath for 10 minutes at 60°C. The sample was extracted using petroleum ether (60°-65c). The Fatty Acid Methyl Ester (FAME) were isolated using gas chromatography. The FAME was injected and separation was carried out on HP capillary column (AOAC, 2000; method no: 969.33).

3.2.6 Alpha tocopherol and total carotene content in selected oils

The α-tocopherol in the oil samples was determined, using the Cecil 505E spectrophotometer at 470 nm. Total carotene was analysed according to AOAC 974.29 type 4 method (2000) using the Methrom Spectronic 21D model spectrophotometer at 328 nm.

3.3 Study Two: Effects of varying levels of dietary oils on performance, serum biochemical indices and calcium retention in broiler chickens

Experimental diet

The test oils used in the feeding trial were Palm Kernel Oil (PKO), Coconut Oil (CO) and Shea Butter (SB) which were selected after their physico-chemical assessment. They were obtained in bulk to avoid variation due to batches. The PKO, CO, SB, were included in the diets at the levels of 1.0, 2.0 and 3.0.

3.3.1 Dietary Layout and Experimental Design

The experimental was a completely randomised design

Treatment 1: Basal diet + 1.0% shea butter

Treatment 2: Basal diet + 2.0% shea butter

Treatment 3: Basal diet + 3.0% shea butter

Treatment 4: Basal diet + 1.0% palm kernel oil

Treatment 5: Basal diet + 2.0% palm kernel oil

Treatment 6: Basal diet + 3.0% palm kernel oil

Treatment 7: Basal diet + 1.0% coconut oil

Treatment 8: Basal diet + 2.0% coconut oil

Treatment 9: Basal diet + 3.0% coconut oil

The gross composition (g/ Kg) of starter and finisher diets fed to broiler chickens is shown in Table 3.1.

3.3.2 Management of broiler chickens

The poultry house and equipments used were thoroughly washed and disinfected before the arrival of the chicks. One-day-old Abor Acres broiler chicks (n=360) were obtained from a reputable commercial hatchery and raised on floor pens in a well ventilated and illuminated standard poultry house. On arrival, the chicks were subjected to ten days of brooding with heat supplied by coal pots andrandomly allotted to nine dietary treatments. The ambient temperature during the experiment ranged from 18-41^{o}c humidity ranged from 30-88. Each treatment was replicated four times and a replicate comprised ten chickens. The broiler chickens had unrestricted access to the experimental diets and water for six weeks. Broiler starter mash was fed to the experimental chicks from one day to fourteenth day. While the finisher diet was given to the chicks from day 15 to day 42. On day 20 post-hatch, the birds were transferred to metabolic cages and randomly allotted experimental diets with 4 replicates. The first 2 days allowed for acclimatization to the experimental diets. All necessary routine management practices were adhered to, dry and friable litter was ensured by evacuating wet portions.

Table 3.1: Gross composition (g/100g) of basal starter and finisher diets fed to broiler chickens

Ingredients (%)	Starter	Finisher
Maize	45.05	57.05
Soyabean meal	42.00	30.00
Wheat offal	8.24	8.24
DL-Methionine	0.20	0.20
L-Lysine	0.10	0.10
Oyster shell	1.00	1.00
Vegetable oil	2.00	2.00
Dicalcium phosphate	0.83	0.83
Vitaminmineralpremix	0.25	0.25
Salt	0.33	0.33
Total	100.00	100.00
Calculated analysis		
ME (Kcal/kg)	3011.91	3112.57
Crude protein (%)	23.09	21.00
Phosphorus (g/kg)	0.52	0.51
Calcium (g/kg)	0.98	0.82

vitamin-mineral premix content in one kg of the diet: Mn, 120mg; Iron,100mg; Se. 0.12mg ,Zinc, 80mg; copper, 8.5mg; Iodine, 1.5mg; Cobalt, 0.3mg;; Antioxidant, 120mg. [2] Limestone contains 38% calcium, 5.5mg; Niacin, 55mg; Calcium pantothenate, 11.5mg; Vit. B6, 5mg; Vit. B12, 0.025mg; choline chloride, 500mg; Folic acid, 1mg; Biotin,0.08mg; Vit. A, 12,500 I.U; Vit. D3, 2,500 I.U; Vit. E, 40mg; Vit. K3, 2mg; Vit. B1, 3mg; Vit. B2, ME- metabolisable energy

3.3.3 Performance characteristics

3.3.3.1 Feed intake (g/bird): was taken weekly and calculated as the difference between feed offered and left over.

3.3.3.2 Feed intake = Total feed supplied – Left over feed in each pen.

3.3.3.3 Weight gain (g/bird): Weekly weighing of the bird was done and records of body weight changes were kept. Weight gain was calculated as the weight obtained after deducting the initial weights from the final weights of broiler chickens.

3.3.3.4 Feed conversion ratio: was calculated as the ratio between weight of total FI and weight gain within the period in each pen

$$\frac{\text{Total feed consumed (g)}}{\text{Increase in weight (g)}}$$

3.3.3 Carcass characteristics

At day 42 of the experiment, two broiler chickens from each replicate with weights closest to the class mean weight were selected, properly tagged and isolated. They were deprived of feed but had unrestricted access to water for eight hours. The chickens were weighed prior to slaughter. The chickens were exanguinated by severance of the jugular vein and hung for total blood drainage. Defeathering was done after immersing the birds in warm water at 70^{o}c for 2-3 minutes and were dissected into primal cuts for carcass yield assessment relative to the live weight.

Organ weights (OW): The full and empty gizzard, spleen, heart and liver of the sacrificed broiler chickens from each treatment were weighed and recorded.

OW was calculated with the formula $= \frac{\text{Weight of organ}}{\text{Live weight of chicken}} \times 100$

3.3.4 Digesta and faecal samples

Between days 21 and 25 post hatch, 2 chickens per replicate were selected and housed in a metabolic cage for determining the total tract digestibility. Fresh faecal samples were obtained everyday from the collection tray placed under the cages and sprinkled with dilute sulphuric acid to avoid contamination by flies and to arrest further degradation before collection. Samples were collected 8:00am daily and were pooled

on replicate cage basis, bulked and stored in a refrigerator at -4°C. Faecal samples were oven dried at 55°C for 5 days in a force-draft oven. On day 42, two broiler chickens per replicate were selected, slaughtered and dissected to obtain digesta from the distal 2/3rd of the ileum using the procedure of (Mutucumarana and Ravindran, 2016). Deionized water was used to flush out the contents while digesta from each relicate bird were merged, and freeze-dried.

3.3.5 Chemical analyses

Dry matter composition was determined by placing one gram each of excreta and ileal samples in dried and pre-weighed crucibles in an oven at 105°C for 24 hours (AOAC, 2005 method no: 930.15). Samples were ashed for phosphorus and calcium determination. Phosphorus in ileal samples were determined by colorimetric assay read at 400 nm after digestion with nitric acid and perchloric acid while calcium was determined by colorimetric assay after digesting with 6.0M HCL to release calcium (AOAC international, 2005; method no: 968.08).

3.3.6 Determination of Nutrient Retention and Apparent Precaecal Digestibility

On day 42, the earlier caged two broiler chickens were slaughtered, and opened up to obtain digesta from the distal two-third of the ileum (Mutucumarana and Ravindran, 2016).

Actual precaecal digestibility and retention coefficients of calcium and phosphorus were estimated on digestibility marker ratios with the following formula:

$$AND\ (\%) = 100 - [(Cr_i / Cr_o) \times (N_o/N_i) \times 100]$$

Where AND= Actual nutrient digestibility for ileal digesta or excreta

Cr_i = Chromium concentration in feed taken

Cr_o = Chromium concentration in ileal or excreta output

N_i = Concentration of nutrient in the diet

N_o = Concentration of nutrient in ileal

3.3.7 Serum biochemical indices

At day 42, blood (5 mL) was sampled from two broiler chickens per replicate using needles and syringes inserted through the jugular vein and poured into non-heparinised

bottles for serum biochemical indices determination. The blood was left to clot and the serum separated using a 90-2 centrifuge (Bosch, China 1991) at 3500 rpm for 10 minutes. Serum enzymes assessed were superoxide dismutase, gluthathione peroxidase, aspartate transferase and alkaline phosphatase (Underwood and Suttle, 2001). Serum minerals such as calcium and phosphorus were also determined (AOAC international, 2000; method no: 984.27). Samples were read at different wavelengths specific for each parameter.

3.3.8 Bone characteristics

Two broiler chickens per replicate were slaughtered and the left thigh removed and immersed in boiling water at about 100^0C for 10 minutes. The thigh was de-fleshed and the left tibiae was removed and defatted. The left tibia was then dried in a force drift oven at 55°C for 3hours, weighed and ashed in a muffle furnace at 600°C for six hours. Percentage ash was calculated relative to dry weight of tibia. Phosphorus and calcium in tibia were determined spectrophotometrically and read at wavelength of 400nm for phosphorus and 520nm for calcium AOAC (2000).

3.3.9 Experimental Design and Model

The experiment was a 3 x 3 factorial arrangement in a completely randomised design. The factors were oil types (shea butter, palm kernel and coconut) and inclusion levels (1.0, 2.0 and 3.0%). The experimental model is as shown below

$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha_i \beta_j + eijk$

Where Y_{ijk} = Observation k in level I of factor A and level j of factor B

μ = Overall population mean

α_i = Effect of i level of dietary oil types

β_j = Effect of j level of inclusion levels

$\alpha_i \beta_j$ = Interaction effect of oil types and inclusion levels

eijk = Random error with mean 0 and variance

3.3.10 Statistical analysis

Data were analysed using descriptive statistics, analysis of variance (ANOVA), and regression using SAS (2012). Means of treatments were separated with Duncan's Multiple range test at α-$_{0.05}$.

3.4 Study Three: Effects of oil types and dietary calcium levels on performance, serum biochemical indices, carcass traits, ileal calcium and phosphorus digestibility in broiler chickens

3.4.1 Experimental diet

Optimum level of dietary PKO, CO and SB inclusion were selected after the first feeding trial and were incorporated into diets at a fixed level of 2.0% with varying levels of dietary calcium at 0.75, 1.00 and 1.25% respectively.

3.4.1.1 Dietary Treatments Layout

The experimental design was a completely randomised design

Treatment 1: Basal diet + 2.0% shea butter + 0.75% dietary calcium

Treatment 2: Basal diet + 2.0% shea butter + 1.00% dietary calcium

Treatment 3: Basal diet + 2.0% shea butter + 1.25% dietary calcium

Treatment 4: Basal diet + 2.0% palm kernel oil + 0.75% dietary calcium

Treatment 5: Basal diet + 2.0% palm kernel oil + 1.00% dietary calcium

Treatment 6: Basal diet + 2.0% palm kernel oil + 1.25% dietary calcium

Treatment 7: Basal diet + 2.0% coconut oil + 0.75% dietary calcium

Treatment 8: Basal diet + 2.0% coconut oil + 1.00% dietary calcium

Treatment 9: Basal diet + 2.0% coconut oil + 1.25% dietary calcium

The gross composition (g/ Kg) of diets fed to broiler chickens is shown in Table 3.2.

3.4.2 Management of Broiler Chickens

The poultry house and equipments used were thoroughly washed and disinfected before the arrival of the chicks. One-day-old Abor Acres broiler chicks (n=550) were obtained from a reputable commercial hatchery and raised on floor pens in a well ventilated and illuminated standard poultry house. On arrival, the chicks were subjected to ten days of brooding with heat supplied by coal pots andrandomly allotted

to nine dietary treatments. The ambient temperature during the experiment ranged from 18-41°c humidity ranged from 30-88. Each treatment was replicated six times and a replicate comprised ten chickens. The broiler chickens had unrestricted access to the experimental diet and water for six weeks. Broiler starter mash was fed to the experimental chicks from one day to fourteenth day. While the finisher diet was given to the chicks from day 15th to day 42. On day 20 post-hatch, the birds were transferred to metabolic cages and randomly allotted experimental diets with 6 replicates. The first 2 days allowed for acclimatization to the experimental diets. All necessary routine management practices were adhered to, dry and friable litter was ensured by evacuating wet portions.

Table 3.2: Gross composition (g/100g) of basal starter and finisher diets fed to broiler chickens

Ingredients (%)	Starter	Finisher
Maize	45.05	57.05
Soyabean meal	42.00	30.00
Wheat offal	8.24	8.24
DL-Methionine	0.20	0.20
L-Lysine	0.10	0.10
Oyster shell	1.00	1.00
Vegetable oil	2.00	2.00
Dicalcium phosphate	0.83	0.83
Vitaminmineralpremix	0.25	0.25
Salt	0.33	0.33
Total	100.00	100.00
Calculated analysis		
ME (Kcal/kg)	3011.91	3112.57
Crude protein (%)	23.09	21.00
Phosphorus (g/kg)	0.52	0.51
Calcium (g/kg)	0.98	0.82

vitamin-mineral premix content in one kg of the diet: Mn, 120mg; Iron,100mg; Se. 0.12mg ,Zinc, 80mg; copper, 8.5mg; Iodine, 1.5mg; Cobalt, 0.3mg;; Antioxidant, 120mg. [2] Limestone contains 38% calcium, 5.5mg; Niacin, 55mg; Calcium pantothenate, 11.5mg; Vit. B6, 5mg; Vit. B12, 0.025mg; choline chloride, 500mg; Folic acid, 1mg; Biotin,0.08mg; Vit. A, 12,500 I.U; Vit. D3, 2,500 I.U; Vit. E, 40mg; Vit. K3, 2mg; Vit. B1, 3mg; Vit. B2, ME: metabolsable energy

3.4.2 Performance characteristics

3.4.2.1 Feed intake (g/bird) was taken weekly and calculated as the difference between feed offered and left over.

3.4.2.2 Feed intake = Total feed supplied – Left over feed in each pen.

3.4.2.3 Weight gain (g/bird): Weekly weighing of the bird was done and records of body weight changes were recorded. Weight gain is calculated as the weight obtained after deducting the initial weights from the final weights of broiler chickens.

3.4.2.4 Feed conversion ratio: was calculated as the ratio between weight of total FI and weight gain within the period in each pen

$$\frac{\text{Total feed consumed (g)}}{\text{Increase in weight (g)}}$$

3.4.3 Carcass characteristics

At day 42 of the experiment, two broiler chickens from each replicate with weights closest to the mean weight were selected, properly tagged and isolated. They were deprived of feed but had unrestricted access to water for eight hours. The chickens were weighed prior to slaughter, and slaughtered through the cutting of jugular vein and hung for total blood drainage. Defeathering was done after immersing the birds in hot water for 2-3 minutes and the carcass after evisceration were divided into primal cuts for carcass yield assessment.

Organ Weights (OW): The full and empty gizzard, spleen, heart and liver of the sacrificed broiler chickens from each treatment were weighed and recorded relative to live weight.

OW is calculated with the formula = $\frac{\text{Weight of organ}}{\text{Body weight of chicken}} \times 100$

3.4.4 Digesta and faecal samples

Between days 21 and 25 post hatch, 2 chickens per replicate were selected and housed in a metabolic cage for total tract digestibility. Fresh faecal samples were obtained daily from the collection trays placed beneath the cages and sprinkled with dilute sulphuric acid to avoid contamination by flies. Samples were collected every morning

were pooled on replicate cage basis, bulked and stored in a refrigerator at -4°C. Faecal samples were subjected to air drying at 55°C for 5 days in a force-draft oven. On day 42, two broiler chickens per replicate were selected, slaughtered and dissected to obtain digesta from the distal 2/3rd of the ileum using the procedure of (Mutucumarana and Ravindran, 2016). Deionized water was used to flush out the contents while digesta from each replicates were merged, kept frozen and freeze-dried.

3.4.5 Chemical analyses

Dry matter composition was determined by placing one gram each of excreta and ileal samples in dried and pre-weighed crucibles in an oven at 105^0C for 24 hours (AOAC, 2005 method no: 930.15). Samples were ashed for phosphorus and calcium determination. Phosphorus in ileal samples were determined by colorimetric assay read at 400 nm after digestion with nitric acid and perchloric acid while calcium was determined by colorimetric assay after digesting with 6.0M HCL to release calcium (AOAC international, 2005; method no: 968.08).

3.5 Apparent precaecal digestibility and retention determination

On day 42, two broiler chickens from each replicate were selected, slaughtered and opened up to obtain digesta from the distal two-third of the ileum (Mutucumarana and Ravindran, 2012).

Actual precaecal digestibility and retention coefficients of calcium and phosphorus were estimated through a method that is based on digestibility marker ratios with the following equation:

$$AND\ (\%) = 100 - [(Cr_i / Cr_o) \times (N_o/N_i) \times 100]$$

Where AND= Actual nutrient ileal digesta or excreta digestibility

Cr_i = Chromium concentration in feed intake

Cr_o = Chromium concentration in ileal or excreta output

N_i = Concentration of nutrient in the diet

N_o = Concentration of nutrient in ileal

3.5.1 Serum biochemical indices

5mL of blood each was sampled and taken from two broiler chickens per replicate using needles and syringes via the jugular vein and poured into non-heparinised bottles for serum biochemical indices determination at day 42. The blood was left to clot and centrifuged immediately at 3500 rpm for 10 minutes to separate the serum. Serum enzymes assessed were superoxide dimutase, gluthathione peroxidase, aspartate transferase and alkaline phosphatase (Underwood and Suttle, 2001). Serum minerals such as calcium and phosphorus were also determined (AOAC, 2000). Samples were read at different wavelengths specific for each parameter.

3.5.2 Bone characteristics

Two broiler chickens in each replicate were slaughtered at 5 weeks, and the left thigh was removed and immersed in hot water of about 100 ^{0}C for 10 minutes. It was defatted and the left tibae was carefully removed, then dried in a force drift oven at 55^{0}C, weighed and ashed in a muffle furnace at 600^{0}C for six hours for determination of tibia ash. Percentage ash determination was calculated as relative to dry weight of tibia. Phosphorus and calcium in tibia were determined spectrophotometrically and read at wavelength specific for each mineral (AOAC, 2000).

3.5.3 Experimental design and model

The design of the experiment was a completely randomised design in a 3 x 3 factorial arrangement. The factors were oil types (shea butter, palm kernel and coconut) and dietary calcium levels (0.75, 1.00 and 2.00%). The experimental model is as shown below:

$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha_i \beta_j + eijk$

Where Y_{ijk} = Observation k in level i of factor A and level j of factor B

μ = Overall population mean

α_i = Effect of i level of dietary oil types

β_j = Effect of j level of dietary calcium levels

$\alpha_i \beta_j$ = Interaction effect of oil types and dietary calcium levels

eijk = Random error with mean 0 and variance

3.6 Statistical analysis

Data obtained were subjected to descriptive statistics, analysis of variance (ANOVA) and regression using SAS (2012). Means were separated with Duncan's Multiple range test at $\alpha_{-0.05}$.

CHAPTER FOUR

RESULTS

4.1 Physico-chemical attributes of selected dietary oils

Physico-chemical characteristics of selected dietary oils is presented in Table 4.1. Different oil sources significantly ($p<0.05$) affected saponification value). It was observed that PKO (104.76±3.21) and CO (109.81±4.93 mgKOH/g) had higher ($p<0.05$) saponification value than GNO (83.6±1.21). The least ($p<0.05$) saponification value was obtained in SHB (60.80±2.87) and SBO (64.15±3.19 mgKOH/g). Iodine value (g/100g) observed in GNO (18.08±0.17) was appreciably ($p<0.05$) higher than in PKO (4.71±0.04), CO (6.95±2.58), SHB (13.91±1.82) and SBO (14.72±0.71). Considerably higher ($p<0.05$) acid value ($KOHg^{-1}$) was obtained in PKO (3.65±0.06) compared to other dietary oils. The least ($p<0.05$) acid value was obtained in SHB (0.90±0.01). Peroxide value observed in PKO (76.73±0.12) was higher ($p<0.05$) when compared with other dietary oils. While, the lowest ($p<0.05$) peroxide value was obtained in SBO (4.98±0.51). Similar ($p>0.05$) peroxide values were observed in GNO (34.85±1.33) and CO (33.52±1.71).

The total carotene (µg/mL) and α-tocopherol content (µg/mL) of different dietary oils is shown in Figure 4.1. It was observed that the total carotene in shea butter (8550.26±12.43) and coconut oil are higher ($p<0.05$) (8345.06±57.79) than palm kernel oil and groundnut oil (4372.67±70.72) while soybean oil (1641.48±72.50) had the least ($p<0.05$) total carotene concentration (1641±72.50). α-tocopherol concentration in Shea butter (53.37±0.16) and soybean oil (51.25±0.15) are similar but were significantly higher than those of groundnut oil (41.45±0.14) and coconut oil (36.66±0.41). The PKO had the lowest ($p<0.05$) α-tocopherol concentration (25.59±0.35).

Table 4.1: Physico-chemical characteristics of the different dietary oils

Oil type	Saponification value (mgKOH/g)	Iodine value (g/100g)	Acid value (mgKOH)	Peroxide value (mEq/kg)
Palm kernel	104.76±3.21[a]	4.71±0.04[d]	3.65±0.06[a]	76.73±0.12[a]
Groundnut	83.626±1.21[b]	18.08±0.17[a]	1.60±0.02[b]	34.85±1.33[b]
Coconut	109.81±4.93[a]	6.95±2.58[c]	1.10±0.03[c]	33.52±1.71[b]
Sheabutter	60.80±2.87[c]	13.91±1.82[b]	0.90 ±0.01[d]	19.36±2.67[c]
Soybean	64.15±3.19[c]	14.72± 0.71[b]	1.60±0.02[b]	4.98±0.51[d]
SEM	5.39	1.34	0.01	6.42

[a,b,c,d] Means of treatments along a column with different superscripts significantly different ($p<0.05$).

SEM – Standard Error of Means

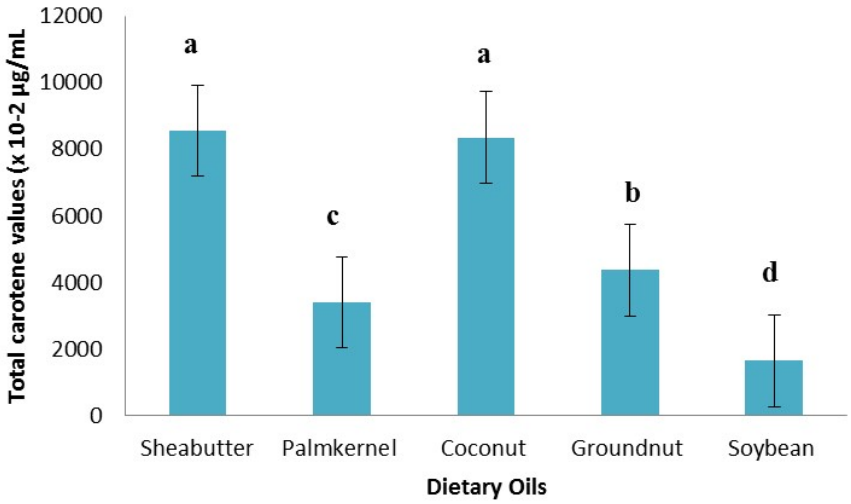

Figure 4.1a: Total carotene contents of different dietary oils

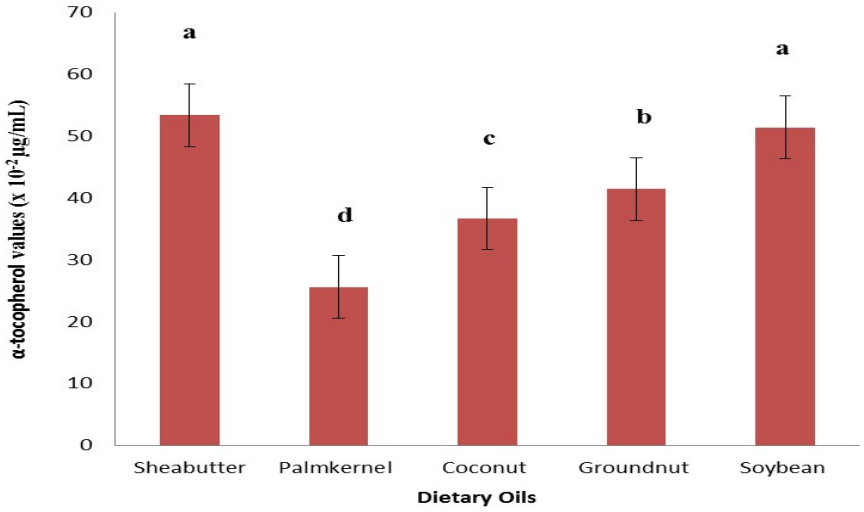

Figure 4.1b: α-tocopherol contents of different dietary oils

4.2 Fatty acid profile of selected vegetable oils commonly used in poultry nutrition

The fatty acid of selected dietary oils is presented in Table 4.2. It was observed that arachidonic acid (%) was significantly higher ($p<0.05$) in SBO (2.33±0.02) than in other dietary oils. The SHB and CO were lower ($p<0.05$) in arachidonic acid concentration (0.08±0.01 and 0.07±0.02) respectively. Behenic acid (%) levels in the DOs ranged from 0.03 (PKO) to 0.06±0.02 (GNO). Higher ($p<0.05$) caproic acid was recorded in SBO (0.28±0.01) when compared with other dietary oils. The concentration of behenic, lauric, linolenic, margaric, oleic, palmitic and stearic acids were substantially greater in groundnut oil in comparison to other dietary oils. Caproic acid concentration in GNO (0.16±0.01), CO (0.28±0.01) and SHB (0.15±0.02) did not differ ($p>0.05$) significantly. The caprylic acid concentration in CO (2.53±0.14) and SHB (2.50±0.02) are higher ($p<0.05$) than other dietary oils. Capric acid concentration ranged from 1.07±0.01 (CO) to 3.74±0.01 (GNO). Erucic acid concentration ranged from 0.04±0.01 (CO) to 0.26±0.01 (GNO). Lauric acid concentration was higher ($p<0.05$) in CO (5.23±0.02), PKO (3.29±0.01) compared to other dietary oils.

The linoleic acid value PKO (18.21±0.03) and GNO (18.05±0.01) are lesser ($p<0.05$) than CO (43.27±0.03) and SHB (19.65±0.02). Higher ($p<0.05$) linolenic acid was recorded in CO (2.83±0.02) compared to other dietary oils. Lignoceric acid ranged from 0.04±0.01 (GNO) to 0.11±0.01 (CO). Margaric acid concentration ranged from 0.20±0.01 (GNO) to 1.06±0.01 (SBO). Myristic acid ranged from 0.67±0.28 (PKO) to 3.79±0.02 (GNO). It was observed that SBO (42.15±0.03) had ($p<0.05$) higher oleic acid concentration than other dietary oils. The oleic acid concentration in GNO (26.95±0.03) and SHB (26.93±0.03) although similar ($p>0.05$) but were lesser ($p<0.05$) compared to other dietary oils. Palmitic acid ranged from 3.54±0.04 (SHB) to 8.83±0.02 (SBO). Higher ($p<0.05$) palmitoleic acid was observed in SBO (0.48±0.14), SHB (0.38±0.01) CO (0.42±0.02) and GNO (0.41±0.02) compared with PKO (0.26±0.02). Stearic acid was higher ($p<0.05$) in SBO (4.07±0.02) than other dietary oils. However, the CO (2.89±0.05) had the least ($p<0.05$) stearic acid concentration.

Table 4.2: Fatty acid profile of different dietary oils

	Dietary oil types				
Fatty acids (%)	Palm kernel	Groundnut	Coconut	Shea butter	Soyabean
Arachidonic	2.10±0.01^{c}	2.23±0.02^{b}	2.25±0.01^{b}	0.08±0.01^{d}	2.33±0.02^{a}
Behenic	0.03±0.01^{e}	0.06±0.02^{a}	0.04±0.07^{d}	0.05±0.01^{b}	0.06±0.01^{c}
Caproic	0.03±0.01^{c}	0.16±0.01^{b}	0.28±0.01^{b}	0.15±0.01^{b}	0.16±0.01^{a}
Caprylic	1.38±0.04^{b}	1.20±0.01^{b}	2.53±0.14^{a}	2.50±0.0.02^{a}	1.36±0.02^{b}
Capric	1.24±0.01^{b}	3.74±0.01^{b}	1.07±0.01^{a}	3.71±0.02^{a}	1.07±0.02^{b}
Erucic	0.06±0.01^{e}	0.26±0.01^{c}	0.04±0.01^{a}	0.24±0.01^{b}	0.08±0.01^{d}
Lauric	3.92±0.02^{b}	1.28±0.02^{d}	5.23±0.02^{a}	1.26±0.02^{e}	2.02±0.01^{c}
Linoleic	18.21±0.03^{c}	19.72±0.01^{c}	43.27±0.03^{b}	19.65±0.02^{b}	18.06±0.02^{a}
Linolenic	2.81±0.04^{c}	0.19±0.02^{d}	2.83±0.02^{b}	0.16±0.01^{e}	3.13±0.02^{a}
Lignoceric	0.06 ±0.02^{b}	0.11±0.01^{a}	0.06±0.01^{b}	0.10±0.03^{a}	0.04±0.02^{b}
Margaric	0.99±0.03^{b}	0.20±0.01^{d}	0.70±0.03^{c}	0.19±0.03^{e}	1.06±0.01^{a}
Myristic	0.67±0.28^{d}	3.79±0.02^{b}	3.67±0.02^{a}	3.77±0.23^{a}	0.77±0.02^{c}
Oleic	38.65±0.02^{a}	26.96±0.02^{b}	5.83±0.02^{c}	26.93±0.02^{b}	42.15±0.03^{a}
Palmitic	5.77±0.02^{b}	3.57±0.01^{c}	8.83±0.14^{a}	3.54±0.01^{c}	8.83±0.02^{a}
Palmitoleic	0.26±0.02^{d}	0.41±0.02^{b}	0.42±0.14^{b}	0.38±0.01^{c}	0.48±0.14^{a}
Stearic	3.13±0.02^{c}	3.55±0.02^{b}	2.89±0.05^{d}	3.51±0.02^{b}	4.07±0.02^{a}

^{a,b,c,d,e} Means of treatments along a row with different superscripts are significantly different ($p<0.05$).

4.3 Effects of varying dietary oil levels on performance, serum biochemical indices and calcium retention in broiler chickens

4.3.1 Performance of broiler chickens fed diets supplemented with different levels of dietary oils

The main effects of dietary oil type and inclusion levels on performance of broiler chickens is shown in Table 4.3. It was observed that dietary oils did not significantly ($p<0.05$) affect by feed intake (g/bird) of broiler chickens and to. The final weight (FW, g/bird) of broiler chickens was notably affected ($p<0.05$) by oil type. The final weight of broiler chickens on CO (942.46) was higher ($p<0.05$) than SHB (903.50) and (894.29) PKO although. The body weight gain (BWG, g/bird) of broiler chickens on CO (906.88) was higher ($p<0.05$) than those on PKO (858.13) but was similar to those on SHB (864.63). However, the feed conversion ratio (FCR) of birds on different oil types were similar ($p>0.05$), and ranged from 2.54 (CO) to 2.69 (SHB). Levels of inclusion of dietary oils significantly ($p<0.05$) influenced performance of broiler chickens. The 2.0% dietary oils inclusion level ($p<0.05$) improved feed intake (2400.26), BWG (925.67) and final weight (964.46) compared to other dietary treatments.

Effect of interaction of oil types and level of inclusion on performance of broiler is shown in Table 4.4.

Interaction of oil type and inclusion level significantly ($p<0.05$) affected performance of broiler chickens. Increase in feed intake was observed in broiler chickens on T_8 (2315.21), T2 (2397.35) and T_5 (2387.13) than other dietary treatments. It was observed that broiler chickens on T_8 had ($p<0.05$) better final weight (998.38) and BWG (959.50) compared to T_7 (FW: 887.43; BWG: 856.75), T_4 (FW: 794.87; BWG: 756.00) and T_3 (FW: 824.88; BWG: 788.13). Lower ($p<0.05$) FCR was observed in broiler chickens on T_1 (2.37) and T_6 (2.38) compared to the dietary treatments, and was similar to those on ($p>0.05$) from T_7 (2.45). **Feed intak**e: SHB at 1% is the same with PKO at 1% but different from CO at 1%. SHB at 2% is the same with PKO at 2% but different from CO at 2%, SHB at 3% is the same with PKO but different from CO at the same level of inclusion.**Final weight**: SHB at 1% is different from PKO and CO at the same level. SHB at 2% is the same with PKO but different from CO. SHB at 3% is

different from PKO and different from CO at the same level. **Weight gain**: SHB at 1% is different from PKO at 1% and CO. SHB at 2% is different from PKO but not different from CO. SHB at 3% is different from PKO and CO. **Feed conversion ratio:** SHB at 1% is different from PKO and CO. SHB at 2% is different from PKO and CO at the same level. SHB at 3% is different from PKO but not different from CO.

Table 4.3: Main effect of oil types and levels of inclusion on performance of broiler chickens

Oil types/levels	Feed Intake	Final weight	BWG	Feed Conversion Ratio
		(g/bird)		
Shea butter oil	2215.78	903.50[ab]	864.63[ab]	2.69
Palm kernel oil	2184.06	894.29[b]	858.13[b]	2.57
Coconut oil	2291.41	942.46[a]	906.88[a]	2.54
SEM	55.14	22.55	21.80	0.06
P value	0.026	0.132	0.283	0.785
Inclusion levels (%)				
1.0	2110.31[b]	881.75[b]	845.71[b]	2.53
2.0	2400.26[a]	964.46[a]	925.67[a]	2.62
3.0	2180.68[b]	894.04[b]	858.25[b]	2.55
SEM	55.14	22.25	21.80	0.06
P value	0.236	0.161	0.025	0.587

[a,b,c] Means of treatments along the same column with different superscripts are significantlydifferent from each other. ($p<0.05$). SEM – Standard Error of Means, BWG- body weight gain

Table 4.4: Interaction effect of oil types and inclusion levels on broiler chicken performance

Oil type	Inclusion level (%)	Feed intake(weekly)	Final weight	Weight Gain	Feed Conversion Ratio
		(g/bird)			
Shea butter	1.0	2190.78^{c}	963.25^{ab}	924.38^{a}	2.37^{c}
Shea butter	2.0	2397.35^{ab}	922.39^{abc}	881.38^{ab}	2.72^{a}
Shea butter	3.0	2127.95^{c}	824.88^{cd}	788.13^{bc}	2.70^{a}
Palm kernel	1.0	2101.68^{c}	794.87^{d}	756.00^{c}	2.78^{a}
Palm kernel	2.0	2387.13^{ab}	972.63^{ab}	936.13^{a}	2.55^{ab}
Palm kernel	3.0	2099.76^{c}	955.38^{abc}	882.25^{a}	2.38^{c}
Coconut	1.0	2099.04^{c}	887.43^{bcd}	856.75^{bc}	2.45^{bc}
Coconut	2.0	2485.11^{a}	998.38^{a}	959.50^{a}	2.59^{ab}
Coconut	3.0	2315.21^{b}	942.88^{ab}	904.38^{a}	2.56^{ab}
SEM		55.14	22.55	21.80	0.06

^{a,b,c,d} Means along a column with different superscripts are significantly different ($p<0.05$)

Relationship between feed conversion ratio to varying levels of dietary oil inclusion in broiler chicken diets

The relationship between inclusion levels of dietary oils and feed conversion ratio of broilers are represented by equations 1, 2 and 3 and shown in Figure 4.2. A positive quadratic relationship was observed between inclusion of SHB and feed conversion ratio as 16% of the observed improvement in FCR was due to inclusion of SHB oil. Similarly, a quadratic relationship was observed between CO and FCR with 3% of the improvement in FCR attributed to CO. However, there was a positive and quadratic relationship between PKO and FCR as 27% of the noted changes in FCR is as a result of PKO inclusion in the diets.

The equations highlighted in the 1, 2 and 3% inclusion levels of the oil samples gave R^2 values which indicated that feed conversion ratio was strongly dependent on inclusion levels of the oils irrespective of the nature of oils. The relationships of FCR and dietary oil inclusion in broiler diets are shown in equations 1, 2 and 3, below and the graphical presentation is shown in figure 4.2

$Y = -0.184X^2 + 0.902x + 1.650$ $(R^2 = 0.16)$ SHB Equation 1

$Y = -0.087X^2 + 0.408x + 2.126$ $(R^2 = 0.03)$ CO Equation 2

$Y = 0.036X^2 – 0.348x + 3.098$ $(R^2 = 0.27)$ PKO Equation 3

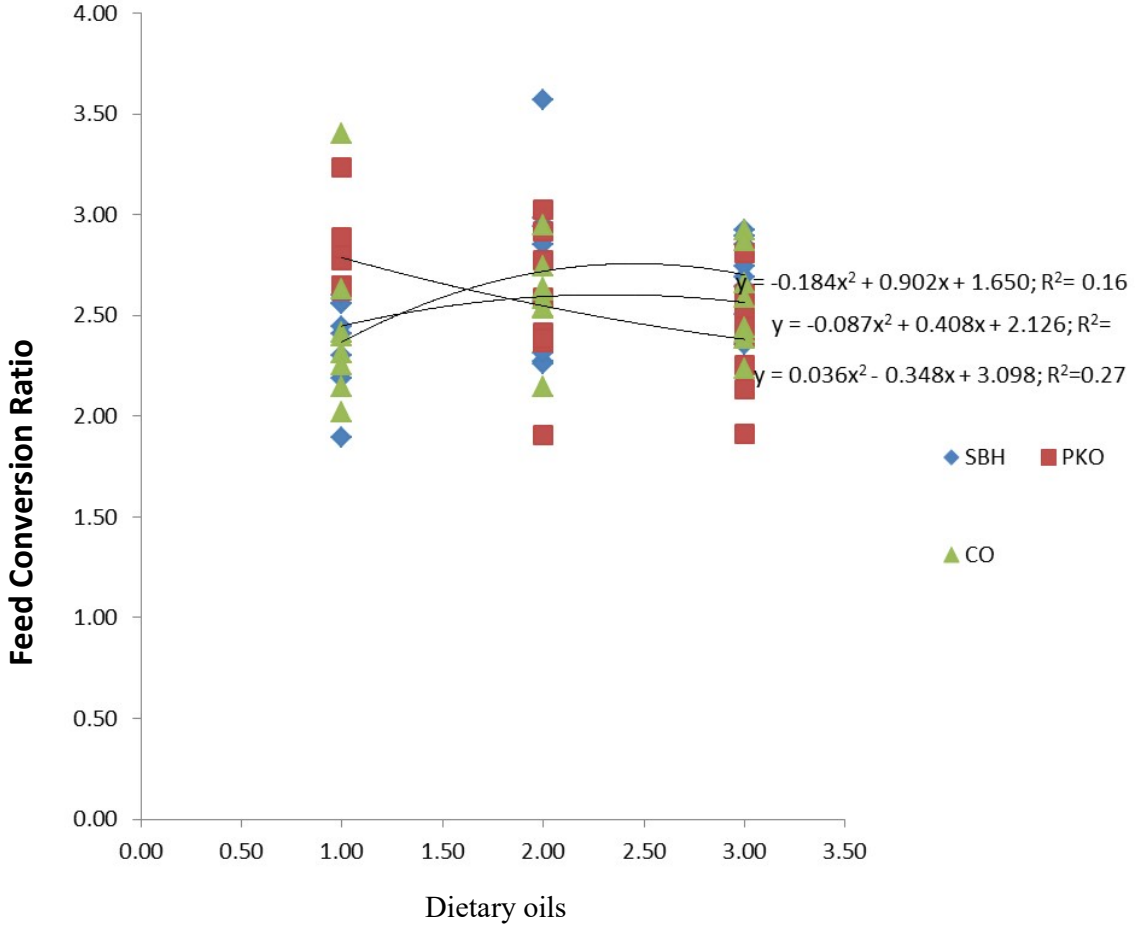

Figure 4.2: polynomial regression of feed conversion ratio on dietary oils in broiler chickens diets

4.3.2 The relative primal cuts of broiler chickens fed different dietary oils

The effects of oil types and dietary inclusion of oil on primal cuts of broiler chickens is shown in Table 4.5. It was noted that live weight of broiler chickens on CO (945.92) is higher ($p<0.05$) them with PKO (862.29) but similar ($p>0.05$) to SHB (903.50). The drumstick was not significantly different ($p>0.05$) with the different oil types and ranged from 10.24 in broiler chickens fed (CO) to 18.29 in broiler chickens fed (PKO). However, the thigh of broiler chickens on PKO (10.35) was higher ($p<0.05$) than other oil types. The weight wings of broiler chickens on CO (8.15) was lower ($p<0.05$) than PKO (9.09) and SHB (8.53). The breast meat as well as the back were not influenced ($p>0.05$) by the dietary oil types. The inclusion of oil up to 3% had a significant effect ($p<0.05$) on live weight (876.46) compared to 2% (953.38) while at 1% (881.88) had no positive influence on live weight. The drumstick ranged from 10.75 (3%) to 17.87 (2%). Increased ($p<0.05$) thigh weight was recorded in birds on 2% (9.91) compared to other inclusion levels of 3% (9.63) and 1% (9.45) levels. The wings were not significantly ($p>0.05$) influenced by different levels of dietary oils and ranged from 8.47 (2%) to 8.73 (3%). Dietary oil levels had significant ($p<0.05$) influence on breast meat of broiler chickens. It was observed that birds on 2% inclusion level (16.25) had highest ($p<0.05$) breast meat value than 3% (15.78) and 1% (15.02) respectively. The back ranged from 13.38 (3%) to 13.81 (2%).

Table 4.5: Relative primal cuts of broilers fed different levels of dietary oils

Oil types/levels	Live weight(g)	Drumstick (g)	Thigh (g)	Wings (g)	Breast meat (g)	Back (g)
Shea butter	903.50[b]	17.64	9.53[b]	8.53[ab]	15.68	13.39
Palm kernel oil	862.29[c]	18.29	10.35[a]	9.09[a]	15.74	13.93
Coconut oil	945.92[a]	10.24	9.11[b]	8.15[b]	15.63	13.32
SEM	24.49	14.11	0.27	0.23	0.42	0.34
P value	0.06	0.31	0.01	0.02	0.98	0.38
Inclusion level						
1.0	881.88[b]	17.59[a]	9.45[b]	8.57[b]	15.02[c]	13.44[b]
2.0	953.38[a]	17.81[a]	9.91[a]	8.47[b]	16.25[a]	13.81[a]
3.0	876.46[b]	10.75[b]	9.63[b]	8.73[a]	15.78[b]	13.38[b]
SEM	24.49	4.11	0.27	0.23	0.42	0.34
P value	0.05	0.39	0.503	0.71	0.12	0.62

[a,b,c] Means of treatment along the same column with different superscripts are significantly different ($p<0.05$) from each other; SEM – Standard Error of Means

Relationship between dietary oils and relative weights of broiler chickens primal cuts of broiler chickens

The relationship between varying dietary levels of selected oils and relative weights of broiler chickens primal is are represented by equations 4, 5, 6, 7 and 8 and shown in figure 4.3. It was observed that with increase in the levels of dietary oils, there was no significant effect ($p<0.05$) on the relative weights of the thigh, wings, and breast meat. The regression of dietary oil and the relative organ weights of broiler chickens is shown in equations 4, 5, 6, 7 and 8 below and is further depicted in Figure 4.3

$Y = -2.228X^2 + 8.142x + 7.392$ ($R^2 = 0.002$) P value = 0.71 Equation 4

$Y = -0.049X^2 + 0.228x + 9.457$ ($R^2 = 0.002$) P value = 0.69 Equation 5

$Y = 0.026X^2 + 0.005x + 8.447$ ($R^2 = 0.01$) P value = 0.46 Equation 6

$Y = -0.089X^2 + 0.583x + 14.98$ ($R^2 = 0.02$) P value = 0.21 Equation 7

$Y = 0.667X^2 – 1.096x + 58.77$ ($R^2 = 0.03$) P value = 0.11 Equation 8

4.3.3 Organ weights of broiler chickens fed diets supplemented with different dietary oil levels

Table 4.6 shows the effect of inclusion level and oil types on organ weights of broiler chickens. It was observed that full gizzard, spleen, heart and empty gizzard were not affected ($p>0.05$) by levels of inclusion. Although, higher ($p<0.05$) liver weight was observed in birds on 2% (3.42) compared to other treatments. Oil types affected ($p<0.05$) the spleen, liver and empty gizzard weights. It was observed that the spleen of broiler chickens on PKO (0.12) was higher ($p<0.05$) than other treatments. Broiler chickens on CO had heavier ($p<0.05$) liver weight (3.45) than broiler chickens on SHB (2.59) and PKO (2.57). The birds on PKO had higher empty gizzard weight (3.35) compared to SHB (2.94), although was not different significantly ($p>0.05$) from CO (3.14).

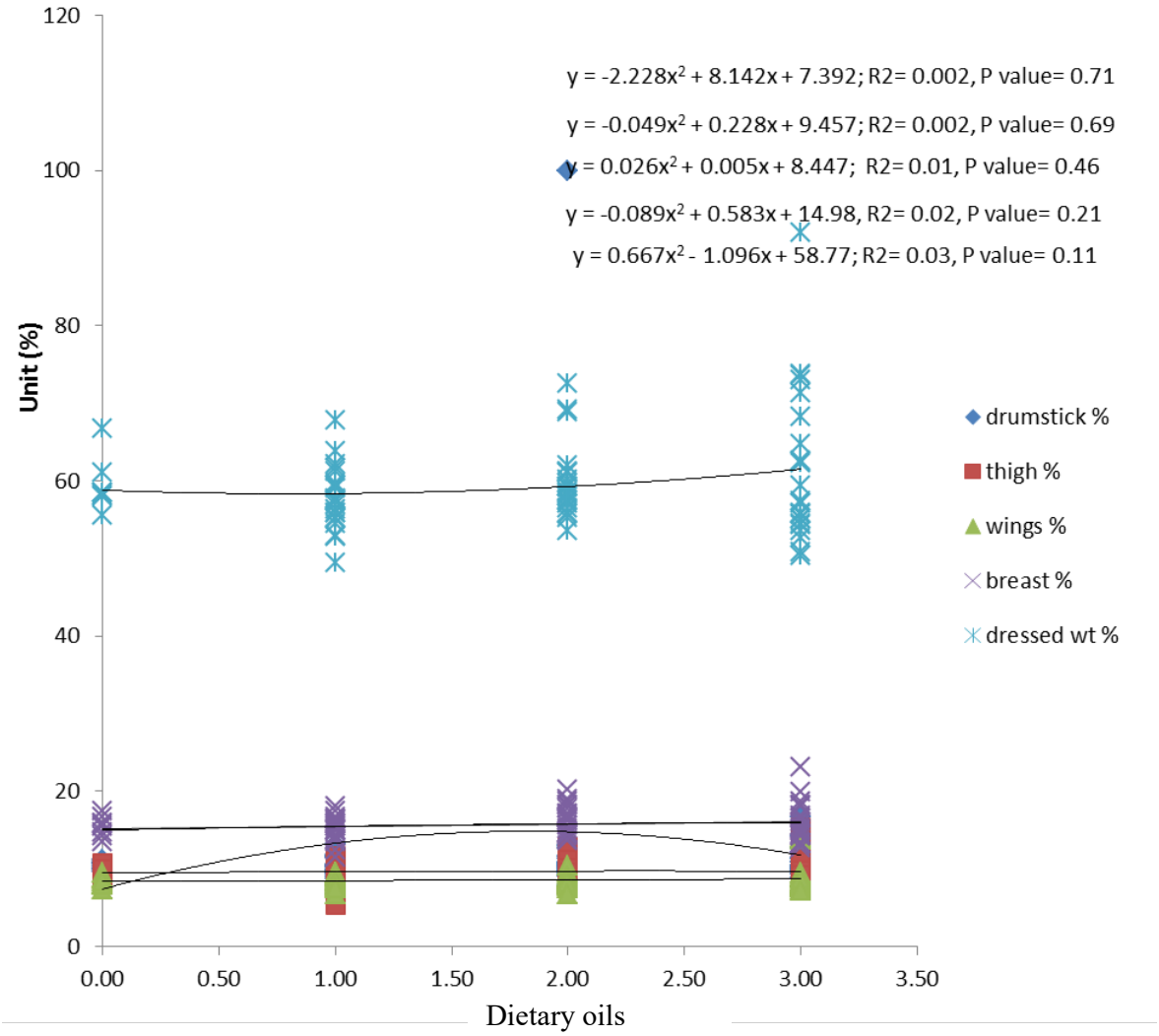

Figure 4.3: Polynomial regression of primal cuts and inclusion levels of selected dietary oils on broiler chickens.

Table 4.6: Relative organ weights (%) of broiler chickens as affected by varying dietary oils

Oil type/ levels	Full gizzard (%)	Spleen (%)	Heart (%)	Liver (%)	Empty gizzard (%)
Shea butter	4.58	0.12[ab]	0.47	2.59[b]	2.94[b]
Palm kernel	5.05	0.12[a]	0.52	2.57[b]	3.35[a]
Coconut	4.86	0.11[b]	0.46	3.45[a]	3.14[ab]
SEM	0.19	0.003	0.025	0.138	0.084
P value	0.34	0.01	0.34	0.001	0.07
Inclusion level (%)					
1.0	4.78	0.12	0.49	2.53[b]	3.16
2.0	4.65	0.11	0.45	3.42[a]	3.04
3.0	5.06	0.11	0.51	2.68[b]	3.23
SEM	0.19	0.003	0.025	0.138	0.084
P value	0.04	0.82	0.31	0.001	0.55

[a,b,c] Means of treatments along a column with different superscripts differed significantly ($p<0.05$). SEM – Standard error of means

4.3.4 Serum lipid and mineral profile of broiler chickens as influenced by different oil types and varying dietary calcium levels

Serum lipid and mineral profiles of broiler chickens as influenced by oil types and different inclusion levels is shown in Table 4.7. Triglycerides concentration was not influenced ($p>0.05$) by different types of oils. The values ranged from 43.44 mg/dL in PKO to 53.14 mg/dL CO. However, varying inclusion levels of calcium increased ($p<0.05$) triglycerides concentration in broiler chickens. Broiler chickens on 3.0% (54.06) inclusion level had the highest value ($p<0.05$) of serum triglyceride than broiler chickens on other dietary oils. Total serum cholesterol level in broiler chickens on PKO (90.03 mg/dL) was higher ($p<0.05$) than other oil types. Broilers on SHB had the lowest ($p<0.05$) total cholesterol (89.28 mg/dL). Broiler chickens on 2.0% dietary oils had higher ($p<0.05$) total cholesterol level of 89.83, than the dietary treatments.

The blood calcium (14.11 mg/dL) and phosphorus 4.13 mg/dL were significantly ($p<0.05$) higher in broiler chickens on PKO than other experimental rations. However, broiler chickens on 1.0% (13.86 mg/dL) and 2.0% (13.79 mg/dL) dietary oil had higher ($p<0.05$) serum calcium compared to those on 3.0% (13.68). However, serum phosphorus of broiler chickens on 1.0% (4.06) dietary oil was significantly ($p<0.05$) higher than in other treatments. Broiler chickens on 3% dietary oil had the lowest ($p<0.05$) serum phosphorus concentration (3.86).

Effects of interaction of oil types and the inclusion levels on serum enzymes of broiler chickens is shown in Table 4.8. The HDL and phosphorus concentration in broilers on 1% PKO were higher ($p<0.05$) than other treatments. Broiler chickens on 1% CO had ($p<0.05$) increased total cholesterol levels, HDL and calcium than other dietary rations. Serum calcium value observed in broilers on PKO at 3% (14.40) was higher ($p<0.05$) than those on other experimental rations. Total cholesterol: SHB inclusion at 1% is different from PKO at 1% and CO at 1%. SHB at 2% is different from PKO at 2% and not different from CO at 2%. However, SHB at 3% was not different from PKO at 3% but different with CO at 3%. HDL: SHB at 2% is different from PKO and CO. SHB at 3% is different from PKO but different from CO. LDL: SHB at 1% is different from PKO and CO. SHB at 2% is different from PKO and also different from CO at the same level. Triglycerides: SHB at 1% is different PKO at 1% also different from CO. SHB at 2% is not different from PKO and CO. SHB at 3% is different from PKO and

CO at the same level. **Calcium**: SHB at 1% is different from PKO and CO. SHB at 2% is different from PKO and CO at the same level. SHB at 3% is different from PKO and CO. **Phosphorus:** SHB at 1% is different from PKO but not different from CO. SHB at 2% is different from PKO and CO while SHB at 3% is different from PKO and CO at the same level.

Table 4.7: Serum lipid and mineral outline of broiler chickens as affected by varying levels of dietary oils

Oil types	Triglycerides	T.CHOL	Calcium	phosphorus	HDL	LDL
			(mg/dL)			
Shea butter	50.14	89.28[c]	13.37[c]	3.73[c]	22.39[c]	33.69[b]
Palm kernel oil	43.44	90.03[a]	14.11[a]	4.13[a]	22.04[a]	34.41[a]
Coconut oil	53.14	89.77[b]	13.86[b]	4.05[b]	22.78[b]	33.36[c]
SEM	6.50	0.01	0.01	0.03	0.01	0.01
P value	0.23	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Inclusion level (%)						
1.0	46.13[b]	89.73[b]	13.86[a]	4.06[a]	22.75[b]	33.39[c]
2.0	46.53[b]	89.83[a]	13.79[a]	3.98[b]	22.82[a]	33.78[b]
3.0	54.06[a]	89.51[c]	13.68[a]	3.86[c]	22.65[c]	34.29[a]
SEM	6.50	0.01	0.01	0.03	0.01	0.10
P value	0.29	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a,b,c] Means of treatments along a column with different superscripts are significantly different ($p<0.05$). SEM- standard error of means, TCHOL- Total cholesterol, HDL-High density lipoprotein, LDL- Low density lipoprotein.

Table 4.8: Effect of interaction of oil types and inclusion levels of dietary oils on serum lipids of broiler chicken

Oil types	Inclusion level (%)	T.CHOL	HDL	LDL	TRIG	Calcium	Phosphorus
		(mg/dL)					
Shea butter	1.0	88.59^{h}	21.83^{g}	32.88^{c}	69.79^{ab}	12.88^{h}	4.32^{a}
Shea butter	2.0	89.32^{f}	22.33^{e}	33.16^{c}	44.57^{bc}	13.10^{f}	4.22^{a}
Shea butter	3.0	89.88^{d}	23.02^{c}	35.00^{a}	36.02^{c}	14.11^{d}	3.62^{c}
Palm kernel	1.0	90.26^{c}	23.21^{a}	33.37^{c}	36.98^{bc}	14.28^{b}	4.33^{a}
Palm kernel	2.0	89.99^{c}	23.02^{c}	34.98^{a}	50.84^{bc}	14.06^{d}	4.09^{b}
Palm kernel	3.0	89.79^{e}	22.88^{d}	34.83^{a}	42.57^{bc}	13.97^{e}	3.92^{b}
Coconut	1.0	90.29^{a}	23.22^{a}	33.87^{b}	31.57^{c}	14.40^{a}	3.51^{c}
Coconut	2.0	90.15^{b}	23.07^{b}	33.16^{c}	44.35^{bc}	14.19^{c}	3.62^{c}
Coconut	3.0	88.83^{g}	22.02^{f}	33.02^{c}	90.44^{a}	13.05^{g}	4.02^{b}
SEM		0.01	0.01	0.10	6.50	0.01	0.03

^{a,b,c,d,e,f,g,h} Means along a column with different superscripts are significantly ($p<0.05$) different . TCHOL- Total cholesterol, HDL-High density lipoprotein, LDL- Low density lipoprotein, TRIG- Triglycerides, SEM- Standard Error of Means

4.3.5 Serum enzymes of broiler chickens fed dietary oils

The effects of dietary oil types and different inclusion levels on serum enzymes of broiler chickens is shown in Table 4.9. It was noted that the superoxide dismutase (SOD) of broiler chickens on CO (170.04mg dL) was higher ($p<0.05$) than in other treatments. The gluthathione peroxidase (GLU) in birds on CO (12.64mg dL) was greater ($p<0.05$) compared to PKO (11.54mg dL) and SHB (11.24). The aspartate amino transferase (AST) of broiler chickens on PKO (22.19mg dL) was higher ($p<0.05$) than other treatments. The alanine amino transferase (ALT) of birds on SHB (17.28mg dL) was much lower ($p<0.05$) than other treatments. However, ALT value observed in birds on PKO (17.60) was higher ($p<0.05$) than other oil types. The alkaline phosphatase (ALP) ranged from 32.65mg dL (CO) to 32.83mg dL (SHB). The high density lipoprotein (HDL) ranged from 22.04mg dL (PKO) to 22.78mg dL (CO). The low density lipoprotein (LDL) ranged from 33.36 (CO) to 34.41 (PKO). Inclusion levels of dietary oils significantly affected ($p<0.05$) the SOD, GLU, AST, ALT, and ALP of broiler chicken. It was observed that broiler chickens on 3% dietary oil had significantly higher ($p<0.05$) SOD 162.00mg dL, AST 21.90mg dL, HDL 22.82mg dL, triglycerides 46.53mg dL, total cholesterol 89.83mg dL and ALP (33.01 mg dL) compared to other treatments.

Effects of interaction of different oil types and inclusion levels of the oils on serum biochemical indices of broilers is shown in Table 4.10. Broiler chickens on 3% CO had significantly higher ($p<0.05$) SOD (174.00) than those in other treatments. The GLU of birds on 3% CO (12.68) was greater ($p<0.05$) compared to other treatments. The ALP and ALT of birds on 1% PKO were higher ($p<0.05$) than in other dietary treatments. Broilers on 1% CO had higher ($p<0.05$) ALP, AST, and ALT values. **SOD**: SHB at 1% is different from PKO and CO. SHB at 2% is not different from PKO but different from CO. SHB at 3% is different from PKO and CO. **GLU**: SHB at 1% is not different trom PKO but different from CO. SHB at 2% is not different from PKO but different from CO. SHB at 3% is different from PKO and CO. **ALP**: SHB at 1% is different from PKO and CO. SHB at 2% is different from PKO and CO. SHB at 3% is different from PKO and CO. **AST**: SHB at 1% is different from PKO and CO. SHB at 2% is different from PKO and CO. SHB at 3% is different from PKO and CO.

Table 4.9: Serum enzymes of broiler chickens fed different levels of dietary oils

Oil types	SOD	GLU	AST	ALP	ALT	HDL	LDL
				(mg/dL)			
Shea butter	153.79^{c}	11.24^{c}	21.45^{c}	32.83^{a}	17.28^{c}	22.39^{c}	33.69^{b}
Palm kernel	159.54^{b}	11.54^{b}	22.19^{a}	32.74^{b}	17.60^{a}	22.04^{a}	34.41^{a}
Coconut	170.04^{a}	12.64^{a}	21.92^{b}	32.65^{c}	17.55^{b}	22.78^{b}	33.36c
SEM	0.80	0.20	0.01	0.01	0.045	0.01	0.01
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<.0001	<.0001
Calcium levels (%)							
1.0	158.79^{c}	11.74^{c}	21.94^{a}	32.31^{c}	17.53^{a}	22.75^{b}	33.39^{c}
2.0	162.00^{b}	11.77^{b}	21.90^{a}	33.01^{a}	17.51^{b}	22.82^{a}	33.78^{b}
3.0	162.21^{a}	11.91^{a}	21.72^{b}	32.84^{b}	17.39^{c}	22.65^{c}	34.29^{a}
SEM	0.80	0.10	0.05	0.01	0.01	0.01	0.01
P value	0.007	<0.0001	<0.0001	<0.0001	<0.0001	<.0001	<.0001

^{a,b,c}Means of treatments along a column with different suprscript differed significantly ($p<0.05$). SOD-superoxide dismutase, GLU- gluthathione peroxidase, AST-aspartate, ALP- alkaline phosphate,

Table 4.10: Effect of interaction of oil types and inclusion level on serum enzymes of broilers

Oil type	Inclusion level (%)	SOD	GLU	ALP	AST	ALT
		(mg/dL)				
Shea butter	1.0	147.75^{g}	11.09^{h}	31.82^{f}	20.97^{e}	16.95^{h}
Shea butter	2.0	160.00^{de}	11.25^{g}	32.08^{e}	21.42^{d}	17.28^{f}
Shea butter	3.0	152.00^{f}	11.35^{f}	33.02^{c}	22.03^{c}	17.61^{c}
Palm kernel	1.0	163.00^{cd}	11.48^{d}	33.33^{a}	22.38^{ab}	17.83^{a}
Palm kernel	2.0	158.00^{e}	11.43^{e}	32.98^{c}	22.04^{c}	17.54^{d}
Palm kernel	3.0	157.75^{e}	11.69^{c}	32.90^{d}	22.15^{bc}	17.43^{e}
Coconut	1.0	165.75^{c}	12.60^{b}	33.33^{a}	22.43^{a}	17.82^{a}
Coconut	2.0	170.00^{b}	12.61^{b}	33.16^{b}	22.23^{abc}	17.68^{b}
Coconut	3.0	174.00^{a}	12.68^{a}	32.03^{e}	21.08^{e}	17.12^{g}
SEM		0.80	0.10	0.01	0.05	0.01

^{a,b,c,d,e,f,g,h}Means along a column with different superscript are significantly different ($p<0.05$). SOD: superoxide dismutase, GLU: gluthathione peroxidase, ALP: alkaline phosphatase, AST: aspartate transferase, T.CHOL: total cholesterol, SEM- standard error of means.

4.3.6 Calcium and Phosphorus retention in broilers fed varying levels of dietary oils

The effect of oil types on Ca and P retention in broiler chickens is presented in Table 4.11. It was observed that different oil types had no significant difference ($p>0.05$) on Ca and P retention in broiler chickens. However, it was observed that 2% (87.18) inclusion of dietary oils increased ($p<0.05$) phosphorus retention in the broiler chickens, also from 3% (60.40) to 1% (77.50). Higher values were reported ($p<0.05$) for calcium retention in broiler chickens fed 1.0% (73.30) and 2.0 % (81.45) dietary oils compared to 3.0% (38.01).

Relationship between three dietary oil and varying inclusion level on digestibility of calcium in broiler chickens

The relationship between inclusion levels of oil and Ca digestibility in broiler chickens is represented by equations 9, 10 and 11 and the graphical representation is illustrated in Figure 4.4. There was a positive quadratic relationship between. The inclusion levels of the different oils and digestibility of Ca as 72%, 2% and 39% of the observed improvement in Ca digestibility were due to level of dietary SHB, CO and PKO inclusion in the diet respectively. Optimum Ca digestibility was observed at 1.8% and 1.5% inclusion of CO and PKO, respectively.

$Y= -3.589X^2 – 11.90x + 108.9 \quad (R^2 = 0.72)$ Equation 9

$Y = 46.75X^2 + 181.5x – 90.21 \quad (R^2 = 0.02)$ Equation 10

$Y = -27.04X^2 + 86.96x + 21.92 \quad (R^2 = 0.39)$ Equation 11

Table 4.11: Calcium and phosphorus retention in broiler chickens fed different dietary oils

Oil type	Calcium (%)	Phosphorus (%)
Shea butter	68.42[a]	76.33[b]
Palm kernel	54.70[b]	67.65[c]
Coconut	69.65[a]	81.10[a]
SEM	6.34	4.79
P value	0.19	0.16
Oil Inclusion level (%)		
1.0	73.30[b]	77.50[b]
2.0	81.45[a]	87.18[a]
3.0	38.01[c]	60.40[c]
SEM	6.34	4.79
P value	<0.0001	0.001

[a,b,c] Means with different superscripts are significantly different from each other, SEM- standard error of means

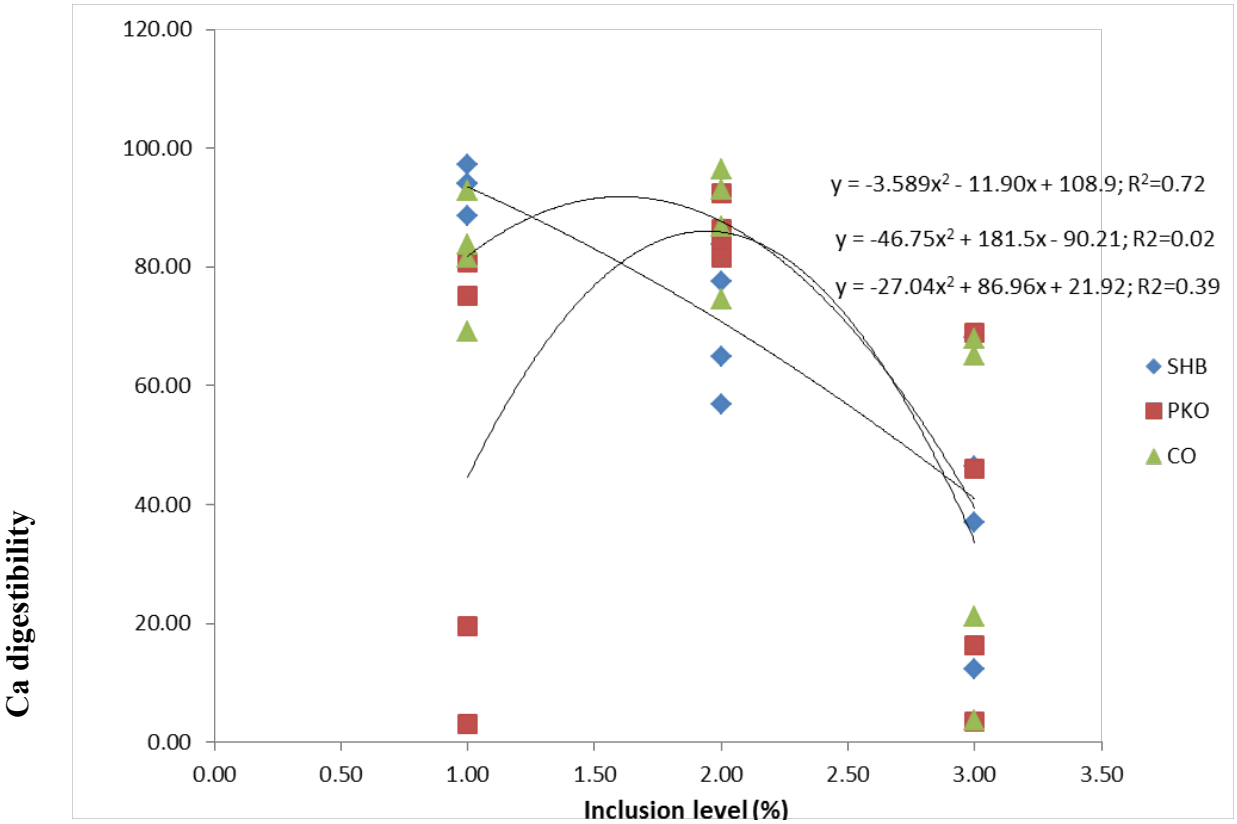

Figure 4.4: Polynomial regression of calcium digestibility and dietary oil inclusion levels in broiler chickens

4.3.7 Ash, calcium and phosphorus retention in broiler chickens as affected by varying levels of dietary oils

Table 4.12 shows the retention of minerals in the tibia of broiler chickens fed dietary oils. It was observed that birds on PKO had higher ($p<0.05$) tibia ash 41.84 than in SHO (31.25) and CO (29.69). Tibia calcium (20.01) and phosphorus (17.03) in birds on PKO were higher ($p<0.05$) than other oil types. The lowest ($p<0.05$) tibia Ca and P retention was observed in birds on SHB.

Inclusion level had influence ($p<0.05$) on tibia Ca and P retention in broiler chickens. It was observed that birds on 3% dietary oil had higher ($p<0.05$) tibia ash (38.85) than in other treatments. Tibia calcium observed in birds on 1.0 % dietary oil was higher ($p<0.05$) in than other treatments. Although, similar calcium retention values were obtained in birds on 2.0% (16.94) and 3.0% (16.89%) dietary oils. Greater ($p<0.05$) tibia phosphorus was observed in birds on 1% (15.76) dietary oil compared with other dietary treatments.

4.4 Performance of broiler chickens as influenced by oil types and levels of dietary calcium.

Table 4.13 shows the performance of broiler chickens that was fed different dietary oils and calcium levels. It was observed that varying dietary calcium (DC) levels did not affect ($p>0.05$) the feed intake of the birds. However, the weight gain (WG) of birds on 1.25% DC (838.58) was higher ($P< 0.05$) than in other treatments. The FCR ranged from 2.61 (1.25% DC) to 2.85 (0.75% DC). Birds on SHB 2052.74 had lower ($p<0.05$) feed intake than PKO 2152.15, but was not significantly different ($p>0.05$) from those on CO 2234.79. The WG was not affected ($p>0.05$) by the different oil types and values ranged from 754.42 CO to 797.69(g/bird) PKO. The FCR of birds on CO (2.96) was significantly ($p<0.05$) higher than in other oil types.

Table 4.12: Tibia ash, calcium and phosphorus retention in broiler chickens as affected by varying levels of dietary oils

Parameters	Tibia	Tibia (%)	
Oil types	**Ash**	**Calcium**	**Phosphorus**
Shea butter	31. 25[b]	15.12[c]	13.49[c]
Palm kernel	41.84[a]	20.01[a]	17.03[a]
Coconut	29.69[b]	18.28[b]	14.11[b]
SEM	1.65	0.10	0.11
P value	<0.0001	<0.0001	<0.0001
Oil inclusion levels (%)			
1.0	33.52[b]	19.58[a]	15.76[a]
2.0	30.40[b]	16.94[b]	14.76[b]
3.0	38.85[a]	16.89[b]	14.10[c]
SEM	1.65	0.10	0.11
P values	0.004	<0.0001	<0.0001

[a,b,c]Means of treatments along a column with different superscripts differed significantly ($p<0.05$) SEM-Standard Error of mean

Table 4.13: Performance indices of broiler chickens fed different dietary oils at varying calcium levels

Calcium levels (%)	Feed intake	BWG	Feed Conversion Ratio
	(g/bird/42 days)		
0.75	2109.72	740.17[c]	2.85[a]
1.00	2142.85	770.56[b]	2.78[a]
1.25	2187.12	838.58[a]	2.61[b]
SEM	48.17	22.60	0.08
P value	0.52	0.01	0.002
Oil type			
Coconut	2234.79[a]	754.42	2.96[a]
Shea butter	2052.74[b]	797.19	2.57[c]
Palm kernel	2152.15[ab]	797.69	2.70[b]
SEM	48.17	22.60	0.08
P values	0.03	0.30	0.05

[a,b,c]Means along a column with different superscript are significantly different ($p<0.05$). BWG-body weight gain SEM- standard error of means.

4.4.1 Serum enzymes of broilers fed different dietary oil types and varying calcium levels

The serum enzymes of broilers on dietary oil types and calcium levels is shown in Table 4.14. It was observed that the SOD value of broiler chickens on CO diets (169.22) was higher ($p<0.05$) than those on other diets. The GLU values in birds on CO (12.26) was significantly higher ($p<0.05$) than in those on other oil types. However, in oil types had no influence ($p>0.05$) on the content of ALP, AST and ALT in the broiler chickens.

It was observed that broiler chickens on 1.25% DC had low level ($p<0.05$) of SOD (158.50) than 1.00% DC (169.00), but was similar to 0.75% DC (162.44). The ALT of broiler chickens on 0.75% DC (17.60 mg/dL) was significantly higher ($p<0.05$) than in 1.00% DC (17.43). However, varying levels of dietary calcium levels had no influence ($p>0.05$) on GLU, ALP, and AST values in broiler chickens and ranged from (11.73, 12.18, 32.55 to 32.84 mg/dL) and (21.65 to 22.56 mg/dL) respectively.

4.4.2 Serum lipid profile of broiler chickens on different dietary oil types and varying calcium levels

The serum lipid profile of broiler chickens on different dietary oil types and different calcium levels is shown in Table 4.15. the total cholesterol value in broiler chickens on CO 89.86 mg/dL and PKO 89.86 mg/dL are similar and higher ($p<0.05$) than SHB (89.47mg/dL). The HDL value observed in broiler chickens on PKO (22.93 mg/dL) was higher ($p<0.05$) than in SHB (22.58). The LDL in PKO (34.21 mg/dL) was significantly ($p<0.05$) higher than other dietary treatments. Serum calcium in broiler chickens on SHB (13.67 mg/dL) was lower ($p<0.05$) than other oil types. However, of oil types had no influence ($p>0.05$) on serum phosphorus and triglyceride values and ranged from 3.91 mg/dL(SHB) to 4.03 mg/dL (CO), and 32.72 mg/dL (SHB) to 33.64 mg/dL (CO), respectively.

There exist a significant ($p<0.05$) influence of dietary calcium of total cholesterol level of broiler chickens fed levels of calcium. Calcium dietary level of 0.75% had Significantly ($p<0.05$) the highest (89.89mg/dL) total cholesterol value which was not significantly ($p>0.05$) different from the chicken fed 1.25% Ca inclusion while 1.0% Ca had significantly ($p<0.05$) the lowest (89.56 mg/dL) total cholesterol.

Table 4.14: Serum enzymes of broiler chickens on different dietary oil types and varying calcium levels

Oil types	Superoxide dismutase	Gluthathione peroxidase	Alkaline phosphatase	Aspartate transferase	Alanine transferase
			(mg/dL)		
Coconut	169.22[a]	12.26	32.76	21.94	17.58
Shea butter	159.33[b]	11.87	32.62	21.69	17.41
Palm kernel	161.38[c]	11.83	32.81	22.62	17.55
SEM	2.79	0.22	0.15	0.46	0.74
P value	0.02	0.09	0.39	1.25	0.06
Calcium level (%)					
0.75	162.44[ab]	12.06	32.81	22.56	17.60[a]
1.00	169.00[a]	12.18	32.55	21.65	17.43[b]
1.25	158.50[b]	11.73	32.84	22.04	17.52[ab]
SEM	2.78	0.22	0.15	0.45	0.07
P value	0.02	0.11	0.13	0.15	0.08

[a,b,c]Means along a column with different superscripts are significantly different ($p<0.05$). SEM- standard error of means.

Table 4.15: Serum lipids of broiler chickens on different dietary oil types and varying calcium levels

Oil types	T.CHOL	HDL	LDL	Calcium	Phosphorus	Triglycerides
			(mg/dL)			
Coconut	89.86[a]	22.83[ab]	33.39[b]	13.81[ab]	4.03	33.64
Shea butter	89.47[b]	22.58[b]	33.64[b]	13.67[b]	3.91	33.22
Palm kernel	89.86[a]	22.93[a]	34.21[a]	13.99[a]	3.99	32.72
SEM	0.13	0.17	0.21	0.14	0.08	0.54
P value	0.07	0.02	0.01	0.00	0.88	0.276
Calcium level (%)						
0.75	89.89[a]	22.93	33.57	13.89	4.03	33.39
1.00	89.56[b]	22.65	33.80	13.59	3.89	33.11
1.25	89.74[ab]	22.78	33.87	13.97	3.99	33.06
SEM	0.13	0.12	0.21	0.20	0.14	0.43
P value	0.05	0.09	0.34	0.24	0.20	0.202

[a,b]Means along a column with different superscript are significantly different ($p<0.05$). TCHOL- Total cholesterol, HDL-High density lipoprotein, LDL- Low density lipoprotein, SEM- Standard Error of Mean

4.4.3 Apparent and total tract digestibility of broiler chickens on different dietary oil types and varying inclusion levels of calcium

The effects of oil types and different levels of calcium on apparent and total tract digestibility of broiler chickens is shown in Table 4.16. Ileal P digestibility was not influenced ($p>0.05$) by oil types and dietary calcium levels. The obtained values ranged from 82.66 g/kg (CO) to 84.59 g/kg (PKO), and 80.88 g/kg(0.75% DC) to 87.95 g/kg (1.25% DC). There exist significant ($p<0.05$) influence of dietary calcium on ileal digestible calcium of broiler chicken fed levels of calcium and oil (Table 4.13). calcium dietary level of 1.25% had significantly ($p<0.05$) the highest (76.73g/kg) ileal calcium digestibility which was not significantly ($p>0.05$) different from the chicken fed 0.75% DC (72.19g/kg) while the chicken fed 1.00% DC had significantly ($p<0.05$) the lowest. (65.48 g/kg). Ileal calcium digestibility of broiler chickens on CO (79.84) was the highest ($p<0.05$) compared to SHB (70.75g/kg) and PKO (64.79 g/kg).

Tibia calcium concentration was not influenced ($p>0.05$) by oil types and dietary levels of calcium and ranged from 92.30 to 98.79 mg/dL and 90.57 to 96.52 mg/dL, respectively. Total tract digestibility of calcium was higher ($p<0.05$) in broiler chickens on 1.00% DC (89.28) compared to other treatments. However, no differences ($p>0.05$) were observed in the effect of oil types. Total tract phosphorus digestibility was not affected ($p>0.05$) by dietary calcium levels and ranged from 78.12% in diets with 0.75% DC to 81.47% in 1.00% DC. However, total tract phosphorus digestibility in broilers fed CO (72.81g/kgDM) is significantly ($p<0.05$) lower with other dietary oils. Tibia ash digestibility of broiler chickens was not influenced by varying dietary calcium but broiler chickens on CO 83.42 and PKO 83.43 significantly ($p<0.05$) increased tibia ash digestibility in broiler chicks compared to those on SHB 80.72.

The relationship between varying levels of dietary calcium on calcium digestibility in broiler chickens

The relationship between varying levels of dietary calcium on calcium digestibility in broiler chickens is represented by equations 12, 13 and 14 and shown in Figure 4.4. The relationships were positive and significant ($P<0.01$) with an optimum calcium digestibility

observed at 0.90% level of dietary calcium when broiler chickens were fed CO. An optimum calcium digestibility was also noticed at 0.87% inclusion level of dietary calcium for SHB and 0.85% dietary calcium level for PKO.

The relationships were explained by the regression equations, 15.0%, 3.00 and 9.00% of the observed improvement in Ca digestibility at 2% of the different oil types were due to the inclusion of Ca in the diet.

$Y = 29001X^2 – 550.0x + 3.270$ ($R^2 = 0.15$) Equation 12

$Y = 36575X^2 – 707.9x + 3.917$ ($R^2 = 0.03$) Equation 13

$Y = -21333X^2 – 403.1x – 1.098$ ($R^2 = 0.09$) Equation 14

Table 4.16: Main effect of oil types and dietary calcium levels on the digestibility of selected minerals

Digestibility	Calcium levels			oil types			P values		SEM
	0.75	1.00	1.25	CO	SHB	PKO	Ca level	Oil type	
Digested Ileal phosphorus(g/kg DM)	80.88	82.04	87.95	82.66	83.73	84.59	0.11	0.87	0.02
Digested Ileal calcium (g/kg DM)	72.19^{ab}	65.48^{b}	76.73^{a}	79.84^{a}	70.75^{ab}	64.79^{b}	0.06	0.02	0.03
Tibia calcium conc. (mg/dL)	94.00	96.52	90.57	92.30	94.59	98.79	0.37	0.37	0.52
Tibia ash (%)	82.84	82.23	82.48	83.42^{a}	80.72^{b}	83.43^{a}	0.29	0.33	0.01
Total tract									
Calcium (g/kg DM)	72.49^{c}	89.28^{a}	81.23^{b}	81.47	82.78	78.85	<0.0001	0.45	0.03
Phosphorus (g/kg DM)	78.12	81.47	80.69	72.81^{b}	83.23^{a}	84.13^{a}	0.59	0.02	0.03

^{a,b,c}Means along a row with different superscripts are significantly different ($p<0.05$), SHO-shea butter oil, CO-coconut oil, PKO-palm kernel oil, SEM-standard error of means

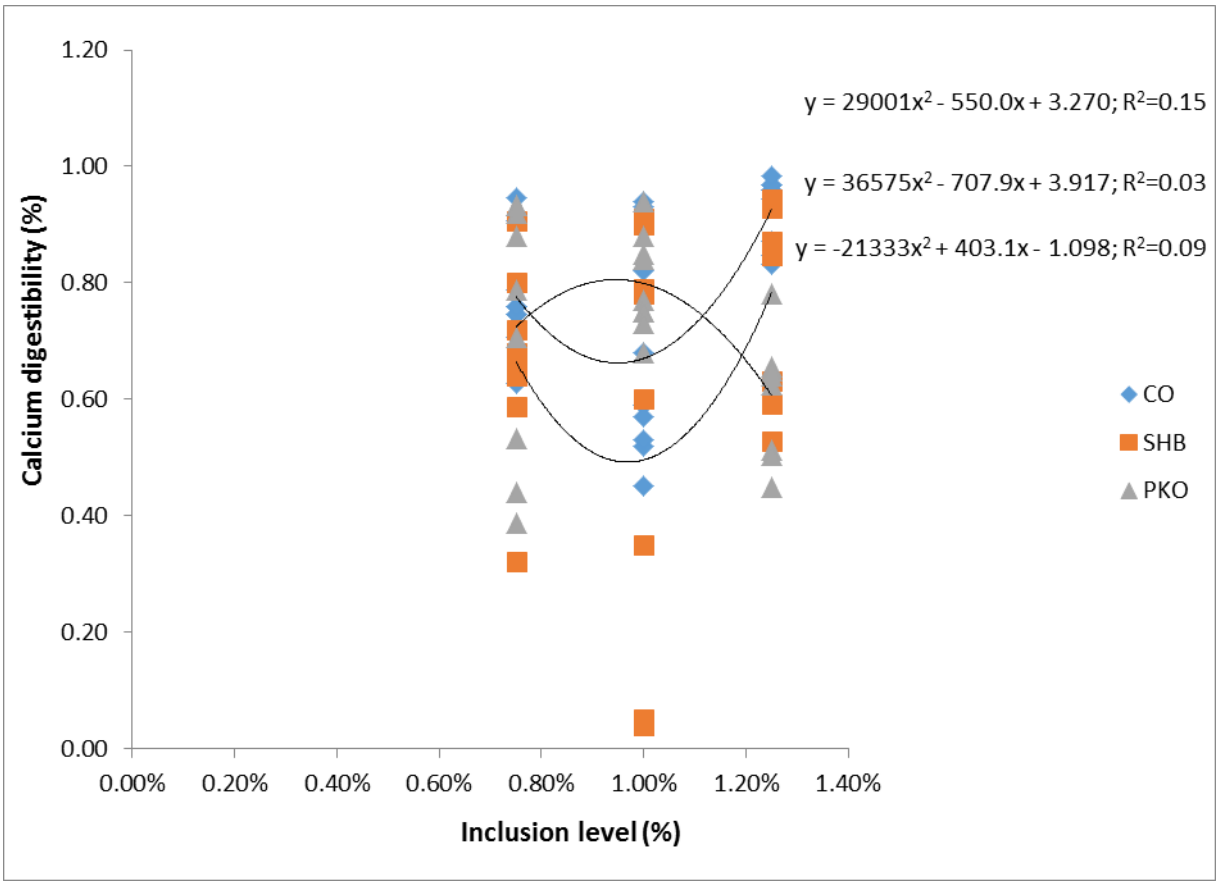

Figure 4.5: polynomial regression of calcium digestibility and varying levels of dietary calcium in broiler chickens

4.4.4 Relative weight of primal cuts of broiler chickens fed different dietary oil types and varying levels of calcium

Main effects of oil types and dietary calcium levels on primal cuts of broiler chickens fed different dietary oil types and varying calcium levels is shown in Table 4.17. The live weight was not influenced ($p>0.05$) by oil types and ranged from 792.89 g in CO to 838.00 g in PKO. The thigh of birds on PKO (27.87g) was significantly higher than those on CO (10.12 g) and SHB (12.10 g). The highest value ($p<0.05$) for the wings was observed in of broiler chickens on (PKO) 8.78g and the least obtained in broilers fed 7.59 g (SHB). The breast meat was not influenced ($p>0.05$) by oil types and ranged from 15.22 g (CO) to 17.08 g (PKO). The back ranged from 11.86 g (SHB) to 13.64 g (PKO). Dietary calcium levels significantly influenced ($p<0.05$) live weight and thigh of broiler chickens. It was observed that broiler chickens on 1.25% DC had higher ($p<0.05$) live weight 877.44 g than other treatments. The thigh 27.86 g of birds on 0.75% DC was higher ($p<0.05$) than other calcium levels. The wings, breast meat and back of broiler chickens were not influenced ($p>0.05$) by either varying levels of calcium and the oil types.

The effect of interaction oil types and dietary levels of calcium on primal cuts of broiler chickens is shown in Table 4.18. It was observed that live weight of broilers on 1.25% Ca and CO (895.75g), 1.25% Ca and SHB (879.00g), 1.00% Ca and PKO (836.92g) and 1.25% Ca and PKO (857.58g) were higher ($p<0.05$) than 0.75% Ca and CO (699.50g), but was not different ($p>0.05$) from 1.00% Ca and CO (783.42 g), 0.75% Ca and SHB (822.17 g), 1.00% Ca and SHB (809.58 g) and 0.75% Ca and PKO (819.50g). The thigh ranged from (9.30 g) 1.25% Ca and PKO to (16.94 g) 1.25% and SHB. The wings of broilers on 0.75% Ca and PKO (10.23 g) and 1.00% Ca and PKO (10.23 g) were higher ($p<0.05$) than other dietary treatments, while the highest value for the breast cut was obtained in broilers fed (18.60) PKO at1.00%DC and least in (13.87) CO at 0.75%DC.

Table 4.17: Main effects of oil types and dietary calcium levels on relative weights of broiler chickens' primal cuts

	Calcium levels			Oil types			P values		SEM
Parameters	**0.75**	**1.00**	**1.25**	**CO**	**SHB**	**PKO**	**Ca level**	**Oil type**	
Live weight	780.39[b]	809.97[b]	877.44[a]	792.89	836.92	838.00	0.01	0.29	23.09
Thigh	28.63[a]	10.32[c]	12.15[b]	10.12	12.10	27.87	<0.0001	<0.0001	16.36
Wings	8.21a[b]	8.64[a]	7.80[b]	8.27[ab]	7.59[b]	8.78[a]	0.29	0.09	0.38
Breast	15.44	16.96	15.89	15.22	16.01	17.08	<0.23	0.13	0.64
back	12.89	13.21	12.52	13.14	11.86	13.64	0.59	0.03	0.48

[a,b,c]Means along a row with different superscripts are significantly different ($p<0.05$). Ca-Calcium, SHO-shea butter oil, CO-coconut oil, PKO-palm kernel oil, SEM- Standard error of means.

Table 4.18: Interaction effect of different oil types and dietary calcium levels on primal cuts of broiler chickens

Oil type	Calcium level (%)	Live weight (g/bird)	Thigh (%)	Wings (%)	Breast meat (%)
CO	0.75	699.50^{b}	10.25	8.60^{ab}	13.87^{b}
CO	1.00	783.42^{ab}	9.92	8.07^{b}	16.05^{ab}
CO	1.25	895.75^{a}	10.20	8.16^{b}	16.03^{ab}
SHB	0.75	822.17^{ab}	9.86	7.90^{b}	16.03^{ab}
SHB	1.00	809.58^{c}	9.50	7.63^{b}	16.25^{ab}
SHB	1.25	879.00^{a}	16.94	7.27^{b}	15.75^{ab}
PKO	0.75	819.50^{ab}	15.76	10.23^{a}	16.42^{ab}
PKO	1.00	836.92^{b}	11.56	10.23^{a}	18.60^{a}
PKO	1.25	857.58^{b}	9.30	7.99^{b}	16.21^{ab}
SEM		23.09	16.36	0.38	0.64

^{a,b}Means of treatments along a column with different superscripts are significantly different from each other ($p<0.05$). SHO-shea butter oil, CO-coconut oil, PKO-palm kernel oil, SEM- standard error of means

CHAPTER FIVE

DISCUSSION

5.1 Physico-chemical characteristics of selected dietary oils

Dietary oils are rich in calorie, supplies energy at a lower cost and a good source of fat soluble vitamins (Chowdhury *et al.*, 2014). Dietary fat enhances the absorption and utilisation of fat soluble vitamins, increases the palatability of the feed, slows down the rate of passage of feed in the Gastro intestinal tract, improve the efficiency of the energy consumed in the diet (Baiao and Lara, 2005). The methods employed in this study proved useful and coherent with reports on stability of some locally available oils (Agunbiade *et al.*, 1999) as well as the understanding of the chemical constituents that inhibits or aids proper digestion and utilisation of oils.The digestion of the oil along the digestive tract could be explained through the chemical properties of each oil. Saponification value is a measure of the volume of alkali needed to neutralise a known amount of oil, it gives an index of the molecular weight of the oil (Praveen *et al*., 2019). The PKO and CO in this study had higher saponification values. It describes that the quantity of alkali required to break the ester bond in the oil to yield more soluble fatty acid were higher than the oils used in this study. Also, saponification values in this study was lower than 253 mgKOH/g and 225 mgKOH/g reported in CO and butter fat by Aremu *et al*., (2006) but higher than 32.54, 31.99 and 34.12 mgKOH/g in rape seed, and niger seed k-7 (Pike, 2003). The saponification value of soybean in the experiment (64.15 mgKOH/g) was close to (65.27 mgKOH/g) for sunflower oil. Oils with high saponification values have been reported to have high levels of lower fatty acids which gives the oil the attribute of ease of

digestibility by the animals. However in digestion, low molecular weight fatty acids aid breakdown and absorption of nutrients easily and prevent insoluble soap formation which jeopardises energy supplied by the oil and mineral retention in birds (Tabeidian, 2010).

Iodine value is a measure of the degree of unsaturation among the fatty acid present in the oil (Aremu *et al*., 2006). Iodine value reported in groundnut oil was higher than in other dietary oils, hence would be better utilised by the chickens. This suggests that the oils would form micelles easily and would be properly absorbed thereby supplying the needed energy and nutrients to the chickens (Wiseman and Salvador, 1991). Foglia *et al*, (1983) reported that iodine value lower than 100 depicts lower degree of saturation, a non-drying oil, slow to oxidation and remain liquid during storage.

Acid value is an important indication of free fatty acid in oils. Higher acid values have been reported to indicate the possibility of sludge formation during digestion, an indication of poor quality of the oil (Shorunke, 1986). The acid values of 1.60mgKOH in SHB and (1.10) in CO in this study were higher than 1.1mgKOH and 0.3mgKOH reported by Duel, (1951) in a study on the acid values of different domestic oils. Peroxide values (PV) however is a measure of the degree to which oxidation has occurred in the oils. The higher the peroxide value the lower the shelf life of the oils (Pearson, 1970). Oils with a high degree of saturation are prone to autoxidation due to the presence of double and triple bonds, and one of the ways to test for autoxidation is the peroxide value. Oxidation that occurs within a fat and oil involves a free radical reaction with oxygen that leads to deterioration of fats and oils that causes bad odours and flavors.

Peroxides are intermediate in the oxidation, as soon as a feed is manufactured it goes through several chemical and physical changes which leads to polymerisation of secondary oxidation products. Undesirable chemical changes occur that affects the odour, colour, nutritional quality and increase in the viscosity of the oil. The PV recorded for PKO 76.73 and GNO 34.85, were higher than other oils and is an indication of a poor shelf quality. Though, peroxide value in this study was greater than 1.70 reported by Aremu *et al*., (2006). The range of acid values of 4.77mgKOH – 12.06mgKOH reported in nine different vegetable oils by Pike, (2003) are higher compared to reports in this study

except for soybean which was comparable to 4.77 and 5.05 reported for palm oil and olive oils, respectively.

The iodine value, acid value, saponification values reported in this study were lower than (41.24, 11.60 and 232.81) g/100g respectively, earlier reported for these parameters by Atasie *et al.* (2009) in palm kernel oil. Dietary oils play a major role in the digestion and absorption of inherent vitamin in oil (Huiling and Carl-Erik, 2004). Fat soluble vitamins are hydrolysed into micelles before they could be transported and utilised *in vivo*. Hence the inability of fat-soluble vitamins to be absorbed is a major challenge in fat digestion.

The different dietary oils in this study had significantly varying levels of α-tocopherol and total carotene contents. This could be as a result of the different chemical constituents, which may be true for SHB and CO which had higher levels of total carotene. Carotenoids are responsible for the diversity of colour in nature, α-carotene, β-carotene and crptoxanthin have demonstrable provitamin A activity (Mukherjee and Mitra, 2009). Provitamin A in oils is readily converted into vitamin A which is needed for vision, growth, cellular differentiation and other physiological functions (Hendler and Rorvik, 2008). Total carotenoids are precursors for vitamin A production that is crucial in modifiable growth, bone metabolism through their involvement in osteoblasts and osteoclasts (De witt *et al.*, 2009). Vitamin A boosts the immune system through antibody production activities. Dietary oil is an ideal option that aids Ca absorption and utilisation by increasing bone health. The higher total carotene in SHB and CO as shown in Figure 1 indicates that the oils are potentially rich sources of provitamin A, while higher α-tocopherol yield observed in SBO and SHBO indicated that SBO and SHB were rich sources of the antioxidants vitamin E.

The oxidation of fat and oils is the cause of deterioration in poultry meat in storage (Brindley, 1984) and it is more sensitive to oxidative damage compared to meat from other sources. It has been documented that muscle membrane in many animals is affected when dietary vitamin E is added to feed which eventually enhanced the quantity of α-tocopherol of the meat (Lauridsen *et al*., 1997). α-tocopherol plays a significant role as a

lipid antioxidant, scavenging of free radicals as well as total carotene which has a great effect in lessening heat stress (Aydin *et al*., 2001).

5.2 Fatty acid profile of the different dietary oils

Fatty acid profile of the oils measures the general state and edibility of the oils. Linoleic, Linolenic and arachidonic acids are essential fatty acids (EFAs) which are metabolically important but birds can not synthesise hence it must be included in their diet (Dvorin *et al.,* 1998). These are obtained in a feed where dietary oil is included. Any deficiency of these EFA has its effect on egg production which could hinder hatchability of eggs. Also, EFA has been documented to minimize growth rates by avoiding its effects in immune stimulation (Balnave, 1971). Results here revealed that the fatty acid components of the oils are of low molecular weight and thus are good for consumption which will be properly utilised by the animal. This corroborates the findings of Jeffri *et al.*, (2010) that FAs inherent in soybean oil and corn oil are USFA with high levels of EFA.

Soybean oil, poultry grease contains a high proportion of linoleic acid, medium proportion of palmitic and oleic acid. Also, tallow contains oleic acid and high levels of SFA mainly stearic acids and palmitic, which reduces its use as a source of energy. In chickens as well as turkeys digestibility of fat is greatly influenced by chain length. The length of the chain length, reduces digestibility and with increase in the number of double bonds, digestibility increases. Thus, the digestibility of vegetable oils is more rapid and effective than that of animal fats (Mossab *et al*., 2000). In a research by Krejci-Treu *et al*. (2009) to find out the fatty acid profile of different oils incorporated in chicken diets as a source of energy and effects on performance and fatty acid compositions in the abdominal fat pad, tallow, had 50% saturated fatty acid (SFA) while in vegetable oils was below 25%. Cotton seed oil values was observed to be high in linoleic acid (55.2%), oleic (20.24%) and palmitic acid to be (20.94%), the values reported here are within the range for cotton oil reported by Pearson, (1970). Also in a similar study by Riaz *et al.,* (2014) it was reported that cotton seed oil contains (50.5%) linoleic acid, (18.3%) oleic and (27.3%) palmitic acid while the ratio of omega- 3 and 6-fatty acids were low. Corn oil was also reported to have a high percentage of Oleic but low in palmitic acids. These values are similar to what Dogan and

Akgul, (2005) reported in some oil seeds he evaluated. He however reported a low linoleic acid amount of less than 2% in argan oil while Adil *et al*., (2017) reported 1% value for linoleic acid. These values were not analogous to what was obtained for the oils analysed in this trial except for sheabutter and CO which had 0.16 and 0.19 respectively which are the least recorded for linoleic acids.

This beneficial effect of oils on the antioxidant profile shows the essence of oil supplementation during hot weather condition, particularly when combined with antioxidant supplementation (Attia and Hassan, 2017). Notably, Piracicaba *et al*., (2009) reported that CO dietary supplementation increases total tocopherols. Tocopherols are essential antioxidants that protect the cell membrane from free radicals (Attia *et al*., 2003). Furthermore, CO is very stable against oxidation and, consequently, not prone to peroxide formation. Therefore, the incorporation of CO enhances the constancy of the feeds (Piracicaba *et al*., 2009). Besides, CO has unique antibacterial, antiprotozoal, and antiviral effects, all of which control microbial rancidity (Enig, 1998). Also, Olomu, (2011) reported that flaxseed oil contains 51% linoleic acid, 59.85% was also reported by Golzar *et al*., (2013) while flaxseed oil had 51% which was the highest. In comparing similar reports, olive oil had the highest oleic acid concentration of 73.13% and the least value of linoleic acid 2.16% compared to values obtained for the oils analysed in this trial.

Groundnut oil is reported to have a value of linoleic acid of 43.27% and the least in sheabutter. When oleic acid concentration is high, omega-3 and omega-6 fatty acids will be low. This difference could be attributed to the different method of analyses as well as the difference in geographical regions where the oils were obtained. The oleic acid level in oil reported by (Atasie *et al*., 2009) for olive oil was 78.1% which was higher than those obtained in this trial. The values of oleic acid ranged from 26.93-42.15 which fell below the range reported by Akgul, (2005) (65-85%) and linoleic acid fell within the range reported by Golzar *et al*., (2013). The difference in the reports could be attributed to the diffrence in the carbon chain lengths and the location.

5.3 Effects of varying levels of dietary oils on performance, serum biochemical indices and calcium retention in broilers

Performance indices of broilers fed different dietary oils

Different dietary oils have been reported to enhance broiler chickens performance (De Groote, 1968; Chowdhury *et al.*, 2014; Dvorin *et al.,* 1998). Baiao and Lara (2005) observed improved performance in broiler chickens fed dietary oils compared with chicks on diets devoid of oils. The advantages of utilising oil in poultry diets is that it increases the absorption of lipoproteins, reduces the heat of metabolism and supplies twice the energy obtained from carbohydrates (Senkoylun *et al*, 1991), and they enhance the absorption of fat soluble vitamins as well as Ca (Liu and Stein 2014). Although, there were similarity in feed intake of broiler chickens fed different dietary oils in this study, broiler chickens on CO supplemented diets had higher body weight gain and final weight. This observation could be due to the medium chain fatty acid nature of CO which has been implicated in enhancing absorption and utilisation of nutrients (Huiling and Carl-Erik, 2004). This result disagrees with the report of Rahimi *et al*. (2011) who fed full fat canola seed to broilers for 42 days and observed no effect on feed intake. The authors attributed this to higher fat content in the diet that reduced fat digestion, absorption and FA synthesis in the birds. Wiseman *et al*. (1991) reported that animal fats such as tallow with high degree of saturation were poorly digested and absorbed by poultry. This is because poultry require large quantities of bile salts to dissolve fat for micellar formation before digestion and absorption.

Although, FCR among broilers fed the different dietary oils were similar in this study which conformed with the report of Viveros *et al.* (2009) who stated that dietary fat sources had no significant effect on FCR, FI, WG of broilers. Moreover, Atteh and Leeson, (1983) stated that the degree of saturation of a fat had a great influence on FI, WG and FCR which also affects utilisation of energy from Polyunsaturated oils. PUFA oils releases more energy and it has a positive effect on performance compared with oil with a high level of SFA. Broilers on diets supplemented with CO had relative improvement in

FCR compared with those on other dietary oils. This could be ascribed to the inherent low molecular weight fatty acids that aided ease of absorption and digestion of nutrients.

Attia and Hassan, (2017) reported oil supplementations improved FCR of broiler under normal and high ambient temperature. (Ferreira, 1999) revealed that 10% CocO increases the rate of production and efficiency. Also, Ayed *et al*., (2015) observed that broilers fed soybean oil present a significantly improved FCR compared and palm oil diets. Besides, Khatun *et al*. (2017) revealed that a 6% PUFA-supplemented diet and a combination of soybean and palm oils significantly enhance the FCR compared to the 6% palm oil diet. On the other hand, Wang *et al*. (2015) reported that broilers fed a CO-enriched diet exhibit no difference in FCR compared to control. (Nobakht *et al*., 2011) concluded that the dietary FO level does not affect the FCR of broilers. Rahimi *et al*. (2011) observed lower weight gain in broiler chickens fed linseed oil compared with those fed palm oil, while Febel *et al*. (2008) observed no effect of different dietary oils on the performance parameters monitored in their studies. Inclusion of oil enhances fatty acid digestibility that has effect on weight gain and FCR in weaned pigs (Liu *et al*. (2014). Conversely, Cera *et al*. (1988) reported no significant influence in weight gain, FCR and the difference could be attributed to the differences in the composition of the diet, inherent constituents of the oil, level of inclusion and the degrees of saturation. However, Garrett and Young (1975) reported that replacing MCFA for soybean oil (SO) for newly weaned pigs enhanced the weight gain and FCR after weaning than those fed diets with tallow or sunflower oil.

Garrett and Young (1975) observed the absorption of saturated fatty acid were more negatively affected than unsaturated fatty acid, thus CO, which is a medium chain fatty Acid are easily digested and absorbed than SFA because they do not require carnitine. Thus medium chain fatty acid are an immediate energy source that can be included in pigs and poultry diet to satisfy their energy demand and reduce metabolic stress as well as fat deposition.

Dietary oils at 2% was more promising than supplementation at 1% or 3%, this observation could mean that supplementing dietary oils below 2% may not be enough to elicit any desirable output on performance of the chickens. However, going beyond this

level may not be desirable as this may be in excess of requirement and thus interferes with digestion and absorption of nutrients and may not be cost effective. The results obtained here corroborates the findings of Baiao and Lara (2005) who reported an improved performance when oil was included in diet of broilers at the first phase of life compared with those that received diets devoid of oil. Also, the results obtained here corroborates the findings of Ekpa, (1995) who fed corn oil and palm oil at an increasing levels of 2, 4, 6, 8 and 10% to broilers. It was reported that there was no significant effects of the oil sources on FCR and weight gain. The observed improvement in performance of broilers in this study could be attributed to the composition of fatty acid which aided micelle formation and proper nutrient utilisation which may not be possible at an increased level.

The degree of unsaturation has been reported to affect the digestion of fat. Sklan *et al*. (1973) observed that utilisation of fat in broilers fed tallow was lower than in birds fed diets containing soybean oil and lard.

According to Balevi and Coskun, (2000) lowest feed intake (FI) was observed in group of birds fed diets containing fish oil 78.03g, 93.3g compared with in those fed tallow and 81.15g in those fed sunflower oil supplemented diets. In a similar study by Newman *et al*. (2002) as well, when different fat blends was fed, the groups fed diets containing tallow and sunflower oils had lower feed efficiency than those fed diet containing only tallow, the difference could be attributed to the low energy supplied by tallow. The FCR were reported to be in between 1.95 and 2.26 which was similar to the FCR values in this study. This is within the range reported by Dvorin *et al.* (1998) the highest weekly weight gain (43.65g) was observed in the group fed diets containing corn oil compared with 37.53g in groups fed fish oil. Also, De witt *et al.* (2009) reported that the live weight of birds fed diets with fish oil were higher than those fed diet supplemented with corn oil, flaxseed, canola and lard. The highest carcass weight was reported in broiler chickens fed diet supplemented with corn oil and the least in the group fed fish oil. Also, Sonaiya, (1988) fed broilers with high and low energy diets and reported carcass weights of between 1585 and1778g at high temperatures of 30°C without reduced in carcass weight with declined temperature.

Effect of interaction of oils and inclusion levels on performance of broiler chickens

Effect of interaction of oils and inclusion levels on performance of broiler chickens elicited positive responses ($p<0.05$), the lowest FCR of 2.37 was observed in broilers on 1% level of SHB. The FCR of 2.38 in chicks fed PKO at 3% level of inclusion was also similar to 2.45 in chicks fed CO at 1% level of inclusion. The highest FCR of 2.78 was in birds fed PKO at 1% compared to other treatment. A lower FCR was observed in broilers was acceptable for optimum production by farmers. It could be observed that the different oils brought out different performance responses in the chicks at different levels of inclusion that could be attributed to the intestinal viscosity of the different dietary oils during digestion. Also, in a study conducted by Zentek *et al*. (2011) when replacing SO with MCFA by 1.4% he reported a positive influence on total tract digestibility which resulted in improved absorption of several nutrients. The MCFAs are considered to be anionic surfactants, and due to this unique characteristic, have antibacterial action. The MCFA can lower the growth of pathogenic strains in the intestine, such as *Campylobacter jejuni, Escherichia coli*, *Salmonella Enteritidis*, (Hong *et al.,* 2012). Dierick and Decuypere (2003) reported that the mechanism resulting in an improved performance in piglets after the addition of MCFA in the diet especially the intestinal lumen. The MCFA has the potential of reducing the population of gut microorganism that compete with the animal for the limited nutrients in GIT.

The result agrees with the findings of other authors (Wiseman and Salvador, 1991, Cascabulho, 2000, Senkoylun *et al.,* 1991), that dietary refined SBO compared with acidulated SBO at varied levels increased linoleic fatty acid of broiler chickens carcass.The improved FCR in chicks on 1% SHB in this study suggests that high total carotenoids and linoleic acid present could be responsible for better utilisation of nutrients in the diets.

5.3.1 Primal cuts of broilers fed different and varying level of dietary oils

Improvement in primal cuts of broiler chickens fed dietary oils have been documented (Baiao and Lara, 2005; Chen and Chiang, 2005). The relative weight of relative primal cut of the broiler chickens varies with the highest live weight in broilers fed CO. Breast

muscle is the most economic or valuable part of broiler chicken (Riaz *et al*., 2014). With the 2% inclusion level of the oil the drumstick, back, thigh and breast had highest weights. Broiler chickens on PKO recorded high thigh and wings weights compared with broilers on SHB and CO. The result obtained here corroborates the findings of Mardhati *et al.* (2011) that a low body weight and FCR values were obtained when unrefined palm kernel oil was fed to broiler chickens compared to soybean based rations. This difference could be attributed to the the major fatty acids which were Lauric and myristic acids that were about 48 and 18%, respectively. This attribute gave CO and PKO their sharp melting properties at room temperature and low unsaturation. Lauric oils were very stable to oxidation with a low degree of unsaturation and their unrefined nature preserved the antioxidant which would have been lost if the oil was to pass through refining. This outstanding property of lauric acid determined their use in feed ingredients as energy source and justified their high price compared to other oils. The polyunsaturated nature of PKO also favoured the development of the muscles involved in locomotion over other body muscles (Baiao and Lara, 2005). The carcass values reported in this work are lower than those reported by Mardhati *et al*. (2011).

Relative organ weights of broilers fed dietary oils

The organ weight changes serve as a sensitive indication of the general health status of an animal. The spleen and liver were the organs influenced by the different dietary oils.The higher liver weight was observed in the liver of chickens fed 2% inclusion of dietary oil. Sanz *et al*. (1999) found no difference in the weight of liver and gizzard of broiler chickens fed soybean and palm oil supplemented diets but reported that heart weight differed with the dietary oils. Abdulla *et al*. (2015) reported no significant effcet of dietary oils on liver, gizzard and heart but observed higher deposition of abdominal fat in palm oil fed broiler chickens.

Some serum indices of broilers fed dietary oils

Aside from triglyceride and LDL of broiler chickens fed PKO which are lower, all other monitored parameters are higher compared with those on other dietary oils. Seyrek *et al*. (2004), observed that cholesterol, LDL and HDL in the serum of birds fed different fat

sources were significantly higher while triglycerides were not affected. A negative relationship was observed between cholesterol and triglyceride values in groups fed sunflower and CO in their diets. Low cholesterol values were reported in the groups fed diets containing sunflower oil (SFO) and high cholesterol noted in those fed CO while triglyceride in the other treatments were lowest. Sacks and Katan, (2002) observed that diets containing different fat sources did not affect blood cholesterol values. Although, HDL was highest in groups fed diets containing CO and lowest in those on tallow. He also reported high HDL in broilers fed tallow while LDL were also high in groups fed tallow and lowest in groups on SFO. Sacks and Katan, (2002) observed that diets containing different fat sources did not affect blood cholesterol values. Though HDL was highest in the groups fed diets containing CO but was the lowest in those fed tallow addition.

Besides, the fatty acid profile of muscle tissue mirrors the dietary lipid profile and can alter the blood levels of lipoproteins and triglycerides (Khatun *et al*., 2017). As a rule, the utilisation of unsaturated fatty acids (UFAs) in poultry diets improves the product quality (for example, ω3 and ω6). This improvement is concordant with the consumers' interest (Zaki *et al.*, 2018) and immune response (Swiatkiewicz *et al*., 2015)

Also, Seyrek *et al*. (2004), reported increased HDL and LDL in groups fed tallow and lowest in broilers fed diet containing SFO. The consumption of PKO improved the utilisation of calcium and phosphorus as observed in the elevated concentrations of these minerals in the serum. The reduced total cholesterol, high HDL and low LDL of broiler chickens on CO suggests that tissue lipases for lipids and cholesterols formation are not mobilised from tissues and this causes a reduction in corticoid secretion which stimulates lipoprotein and aids digestion of the oil in order to release energy (Fritsche *et al*., 1991). Effect of inclusion of oils up to 2% was more promising as serum minerals and HDL were better absorbed while going beyond 2% resulted in lower serum minerals, elevated LDL and high deposit of triglycerides. The reduction in serum minerals could be due to insoluble soaps formation at higher levels of oil inclusion thereby preventing the absorption of minerals as posited by (Loppez-Ferrer *et al*., 1999).

In a study conducted by Febel, *et al*., (2008) no significant influence was observed between the turkey groups concerning the triglycerides, urea, glucose, total and HDL cholesterol in a another study, broilers fed SFO or linseed oil were reported to have lower plasma cholesterol compared with groups fed diets containing tallow (Crespo and Esteve-Garcia, 2002). Seyrek *et al*. (2004) reported that types of added fat could influence metabolism of glucose and it's transportation from plasma to tissue.

Effects of interactions of oil type and level of oil inclusion on serum indices

Serum enzymes are an essential index for wellbeing of animals. The changes in serum parameters from the normal range reported for broilers can reflect the whole body status of an animal. The lower concentration in the plasma of broilers, indicated a better nutrient utilisation after MCFA treatment, which was related to a more better energy production by MCFA (Zentek, 2011). Brindley *et al*. (1984) reported good nitrogen retention which may occur when the dietary energy is increased through utilization of the digested fat, particularly when a low protein broiler diet is fed and they are malnourished due to GIT malfunctioning and low feed intake. There were variations across the different test diet combinations. The highest value for total cholesterol was obtained in broilers fed PKO at 3% while the least was obtained in SHB at 1%.

The serum calcium concentration was highest for broilers on CO diet with 1% inclusion level and the least obtained in SHB at 1% inclusion. Serum P retention was highest in SHB at 1% and least in coconut oil at 1%. The triglyceride concentration varied among the treatment with broilers on 3% CO having the highest concentration. This result suggests that the different oil types had influence on serum enzymes with birds on CO having the highest values. This may be attributed to the medium chain nature of CO that aids micelles formation and absorption of nutrients easily as earlier reported (Gopala Krishna *et al*., 2010).

The different dietary oil had effect ($p<0.05$) on the SOD of broilers, with birds on CO having the highest SOD value while broilers on SHB was least.

CO is a rich source of saturated fatty acids they comprise about 90% of the total fatty acid content. Medium-chain fatty acids (MCFA, C6-C12) represent 60% of the entire fatty acid content (Piracicaba *et al.,* 2009). These fatty acids are absorbed directly into the portal circulation without re-esterification in the intestinal cells (Ferreira 1999). MCFA are burned exclusively and rapidly for energy production (Rubin *et al*., 2010). In contrast, the LCFA are deposited in the adipose tissue (Rego *et al*., 20120). MCFA reportedly decrease fat deposition in the meat (Takeuchi, 2006) and enhance blood lipid profiles in humans (Xie *et al*., 2013) and rats (Han *et al*., 2013). The varying inclusion levels followed a similar trend but at 3% level of inclusion the highest value was obtained, this could be as a result of the scavenging activities of SOD. The birds may be challenged as a result of the test ingredient that increased the activities of SOD and the inherent α-tocopherol content of CO. Habibian *et al*, 2014 reported that vitamin E supplemented to diets at a level of 200 mg kg^{-1} diet increases the response of primary and secondary antibodies in broilers subjected to high temperatures and besides the increase in antibody responses, there was a decrease in the heterophil/lymphocyte ratio, reduced HDL and increased LDL in broiler under heat stress, supplemented with vitamin E at 250 mg/kg feed, with or without the combined addition of organic selenium, showing the pronounced effect of this vitamin.

Due to its soluble nature, vitamin E is deposited at the membrane level, known for its lipoprotein constitution and may perform at this site its best-known action, the antioxidant. Moreover, it can be attached to circulating chylomicrons favoring the immune response to stress in vivo (Voljč *et al*., 2011). As it is a potent scavenger of free radicals, high doses of vitamin E can provide stability to fat deposits, improving the resistance of fresh meat and meat products to oxidation (Dikeman, 2007).

This result negates the findings of Chew (1995) who observed no antimicrobial effect in the serum of birds when they fed essential oil. The (SOD) is the most potent antioxidant and the first detoxifying enzyme in the cell. As an endogenous antioxidant, it acts as a part of the first line of defence against reactive oxygen species (Surai *et al*., 2003). Oxygen

free radicals plays a beneficial and a deleterious effect in animals. The balance between the toxic radical generation and tissue antioxidant status is the outcome of disease. However, the concentration of the peroxide and hydroxyl radicals is far more than what could protect the cells and could result in damage to the endogenous lipids, DNA and proteins. Polyunsaturated (PUFA) rich oils undergoes peroxidation easily leading to free-radical related damage to cells due to an increased production of alkane, in free-radical induced peroxidation of linoleic acid which is the product of peroxidation in lipids (Berger *et al*, 1991). In a study by Berger *et al*. (1991) when pigs were fed an MCFA oil in their diet, a decreased plasma malonyldehyde concentration was reported. MCFAs are very stable to oxidation because they do not contain unsaturated fatty acids which enhances the health status of birds when stressed.

Glutathione peroxidase is an important intracellular enzyme that breaks down hydrogen peroxide to water and peroxides of alcohols in the mitochondria and in the cytosol (Seyrek *et al*., 2004). They function more by inhibiting lipid peroxidation process and thereby protect oxidative cell damage (Jamroz *et al*., 2005). The result obtained for Glutathione peroxidase followed the same trend with broilers on CO having the highest value and birds on SHB had the least. The inclusions of the oils were significantly affected by the activity of Glutathione peroxidase which increases as the oil level increases in the diet. This could be linked to the protecting activities of cells from oxidative damage against detoxification of peroxides in living organisms (Mitruka and Rawnsley 1981). MCFA such as capric acid has been reported to be effective in curbing *Campylobacter jejuni*. They are sources of essential fatty acids and lipophilic vitamins (A, D, E and K), it promotes a sense of satisfaction in its slowing down effects on gastric emptying and reduction of the bioavailability of carbohydrates and enhances the taste, smell, and texture of foods (Lehninger, 2000).

The AST, and ALT are important liver function enzymes and their concentration in the blood are used as indicators of damage to important organs such as the kidney, liver and intestine while ALP measures the integrity of kidney, heart and bone health (Kim and Wyckoff, 1990). Concentrations of these enzymes however were within the normal serum

range for chickens as reported by Mitruka and Rawnsley (1981). The antioxidants enzymes increased with increasing levels of dietary oils while the liver function enzymes and ALP reduced at 3% level of oil inclusion. This observation suggests that dietary oil inclusion increases the activity of the antioxidant enzymes while the integrity of the concerned organs was improved as evident in the low concentrations of ALP, ALT and AST in the serum.

Youssef *et al*. (2020) reported that AST, ALT, and ALP were significantly lower in chickens fed the CO-enriched diet compared to those of the other oil groups. Furthermore, broiler chicks fed FO and MTO presented considerably decreased plasma AST activity compared to the canola groups and raised ALP compared to the CO group. These results indicate a beneficial effect of PUFA on hepatic cell membrane integrity that might be due to enriched phospholipids as an essential part of cell membrane integrity containing two hydrophobic LCFA (Poorghasemi, 2015). The beneficial effects of CO on liver leakage enzymes as previously said might be due to its antioxidant (Piracicaba *et al*., 2009) and antimicrobial effects (Enig, 1998). In literature, oil supplementation improved liver function (Attia and Hassan, 2017). However, Yildirim *et al*., (2014) found that dietary fat sources, including cocoa butter, did not affect plasma AST, ALT and ALP in rats. The differences among the studies might be due to the type of diets, levels and the kind of lipids, and strain of animals. Also the reduction in the activity of AST and ALP observed in this study could lead to a reduction in the activities of bone resorbing cells since Ca and P balance had been the main minerals that aids bone formation.

The effect of interaction of different dietary oils and levels of oil inclusion on serum indices of broiler chickens

The effect of interaction of different dietary oils and levels of oil inclusion on serum indices of broiler chickens revealed that the antioxidant enzymes were more pronounced in CO at 3% level of inclusion while AST, ALT and ALP were higher at 1% level of inclusion. This was followed by PKO and shea butter. These values however fell within

the acceptable physiological range for chickens as reported by (Mitruka and Rawnsley, 1981).

Calcium and Phosphorus retention by chickens fed different dietary oils

The Ca and P retention by chickens fed different dietary oils was similar while retention of these minerals were lowered with increased dietary oil levels. Broilers on CO had the highest retention of Ca and P, while birds on PKO had the least. Calcium plays an indispensable role in skeletal development, growth and bone functions of the animal (Mutucumarana and Ravindran, 2016). The pH range in the (GlT) has effect on the absorption and digestion of nutrients. Most of the Ca salts, except calcium oxalate, are probable to dissolve in the stomach, calcium probably does not stay in solution in the intestine, which is its main site of absorption. The conditions of the small intestine are especially relevant, since insoluble calcium compounds cannot diffuse through the brush border membranes of enterocytes and therefore cannot be absorbed.

Report by Jahanian *et al.* (2008) stated that the digestibilities of the organic dietary ingredients are decreased with increased intestinal viscosity which is likely to reduce the absorption of minerals due to increase in the concentration of potential complexing agents. Jahanian *et al*, (2008) also reported that absorption of minerals could be influenced through a reduced rate of diffusion like the digestion of glucose. The pH of the GIT has a great effect on the solubility of minerals (Shafey and McDonald, 1991) because an increase in pH will have effect on digestibility of minerals because minerals are absorbed as ions and soluble complexes. Also, the retention time has a great effect on mineral absorption and digestion. In a study conducted by Van der Klis *et al*. (2002) on the indigestible but soluble polysaccharide was used to manipulate the pH of the GIT and the duration the digesta stayed in the ileum was recorded. The different condition was achieved by exchanging the methyl cellulose based on the weight of the broilers and keeping the changes observed in the composition of the diet to the lowest level. The effect of the methyl cellulose on minerals was reported to be related to the prevailing conditions in the GIT which was not taken into consideration in this current study. This observation showed that the absorption and utilisation of minerals were not impeded by the type of

oils while going beyond 2% dietary oils in broiler chickens lowered their retention. Observation from this study was different from the reports of Abdulla *et al*. (2017) who reported differences in apparent phosphorus digestibility though calcium was unaffected. The inclusion of the oil at 2% had the highest value across the treatment. This suggests that addition of the oil beyond 2% may not be economical. In the breakdown of fat, two of the fatty acid from the triglycerides are liberated (Senkoylun *et al.,* 1991). These FA have the possibility of binding with minerals such as Ca and Mg forming soluble and insoluble soaps in the gut. If the end product of the digestion is insoluble, both FA and the mineral would not be accessible and utilised then it will be voided as faeces.

Dietary inclusion of oils could enhance minerals digestibility especially Ca and P. Liu *et al*, (2000) reported that inclusion level of oil broilers diets had no influence on digestibility of minerals, weight gain and feed efficiency and the difference in this results may be due to the composition of the diet, the difference in sources of the oil as well as the inherent constituents of the oil which include the saturation degree and the level of inclusion of the oil. The different dietary oils significantly affected Ca and P retention in the tibia of broilers. Increased tibia ash, Ca and P were observed in broilers fed PKO while 3% inclusion of dietary oil favoured ash deposition in the tibia of broilers. The Ca and P were better retained at 1% level of inclusion and decreased with increase in the level of the oil. This could be attributed to the fact that a percentage increase in oil reduced the absorption of minerals thereby leading to insoluble soap formation. This result negate the findings of Leeson *et al*. (1995) that an elevated absorption of Ca was observed when high level of SBO was fed to broilers.

Body weight gain of broilers fed varying levels of DC was significantly increased ($p<0.05$). The BWG was improved with increasing dietary supplementation of Ca with broilers on 1.25%DC having the highest weight gain. This could be attributed to the optimum utilisation of oil at an increased level of Ca which corroborates the findings Shafey and McDonald, (1991), who reported a higher weight gain with increase in the level of calcium these findings, going a step higher than NRC recommendation increased body weight gain irrespective of oil sources.

The FCR followed a similar trend with broilers on 0.75 DC and 1.00% DC having close values. The increased FCR and BWG observed could be attributed to the acceptability of the diets which made chickens to consume more and utilise the feed more efficiently. The different oil types did not influenced the weight gain of the birds. while FI was improved with broilers on coconut oil, then SHB and least in PKO diets. This might due to efficient supply of essential fatty acid especially linoleic acid in CO. The result supports the findings of Mohammed and Horniakova (2011) that herbs, spices and several extracts from plant have appetite and digestion accelerating properties and antimicrobial effect.

Blood is a crucial index used in measuring the physiological status of an animal (Mitruka and Rawnsley 1981). The SOD is an enzyme that catalyses the partitioning of radicals into their ordinary molecular oxygen. There was no observed influence of DC and oil types on the serum parameters measured except for SOD which was the highest in BC on CO than those on other test oils. This may suggests that the birds were not challenged, but the observed increase in the activities of SOD in chickens on CO may be attributed to the presence of lauric acid as well as increased inherent α-tocopherol in CO which exhibited antimicrobial nature in the physiological system of the birds. Results here was contrary to earlier reports by Rose, (1997), who stated that dietary supplementation of Vit. E does not have a significant effect on the antioxidant status in the blood of broilers fed rapeseed oil. Also in a study, Dierick *et al*. (2003) stated that dietary supplementation of vit. E increased SOD activity in the blood of birds fed low-quality oil. The highest activities of SOD was found in broilers on 1.00% DC which was the NRC recommended range and this agrees with (Dvorin *et al.,* 1998), assertion that increased calcium level would not cause differences in Vitamin E levels in the blood or liver of birds fed rape seed oil.

The LDL, HDL and total cholesterol calcium in the serum were influenced by the different oil types with chickens on PKO having high values for these measured parameters. Serum Ca and P were not influenced by either oil types or DC inclusion which could be as a result of negative effects of essential oil on blood parameters which has been reported by (Hong *et al*., 2012). Mohammed and Horniakova, (2011) stated that replacing SFA with PUFA reduced LDL and HDL concentration ratio. While replacing SFA (myristic and lauric) with carbohydrates decreased both LDL and HDL values. However, a negative

relationship obtained here could be as a result of varying levels of individual fatty acid content of the oil which could hinder or aid calcium absorption. Jamroz *et al.* (2005) indicated no effect of polyunsaturated fatty acid on apparent digestibility of nutrients which may hinder Ca and P absorption. The interaction of Ca with oils in the GIT with bile acids could give a clue about the effect of high intake of Ca on serum enzymes. Increase in the excretion of bile acids is directly linked with the formation of Ca complexes that increases the hepatic cholesterol for restoration of bile acids, which eventually reduced cholesterol. A reduction in hepatic cholesterol increases the LDL receptor aiding LDL uptake from circulation and reduced the cholesterol fraction in the blood. High Ca intake reduced the intestinal absorption of cholesterol.

5.4 Effect of oil types and dietary calcium on mineral retention and absorption on tibia bones of broilers

Changes in tibia Ca and P are modulated by high and low affinity Ca-binding sites under the regulation of osteoblast and osteoclast cells (Bronner and Stein, 1995). In a study by Liu *et al.,* (2000) it was observed that low calcium concentrations in the diet led to poor bone development and thin shelled eggs in the layer hen. Results here were contrary to the these previous reports of (Liu *et al.,* 2000). The observed increase in tibia calcium with increase in the DC level could be linked to increased absorption and utilisation of minerals and nutrients in the diet with oil inclusion. An increase of 0.25% DC has been achieved to take care of effective bone formation and strength. Similar observation was noted with DO types on tibia ash and tibia calcium concentration, though with no observed difference. Zollitsch *et al*. (1997) observed no significant influence on bone weight, tibia ash with varying oil level in the diet of chickens. The relationship between Ca and P has been studied (Morrissey and Wasserman, 1971). Excess dietary inclusion of Ca or P forms insoluble calcium phosphate in the intestine. The increased Ca in the serum and a reduced tibia ash concentration is an indication that excess Ca absorption could not be utilised in bone tissue alongside a counter ion, (Shafey and McDonald, 1991).

The digested ileal P followed the same trend, there was no observed difference ($p>0.05$) as the P retention ranged between 80.88g/kg and 87.95g/kg with varying DC. The digestible ileal calcium was affected by varying DC with broilers on 1.25% DC having the highest

retention of Ca the increase in DC level had led to a reduction in the digestible P which could be due to the variation in the Ca:P that has been altered with respect to the different DO. The results obtained here was contrary to the findings of Van der Klis *et al*. (2002) who reported a negative effect on apparent nutrient digestibility with high Ca concentrations which could be due to increase in the pH at high level of Ca concentration and a high acid binding capacity of limestone, which contained mainly Ca (Jahanian *et al.*, 2008). A unit increase in additional Ca with limestone as a component could lead to increase complex formation insoluble Ca-phytate by high pH of the gut.

In providing clue to absorption of calcium in this study, Abdullahi *et al*. (2017) examined the link between providing five varying levels of calcium diet with oil to a set of birds on ileal digestibility and coefficient of nitrogen, P and total tract digestibility of Ca and P. They reported increased DC decreased absorption of P irrespective of the type of oil added to the diet. From the current study, a strong quadratic relationship was observed between ileal digested P and Ca for the varying levels of Ca which was a major constraint for the use of regression methods. From the regression graph, increased Ca inclusion did not favour Ca and P retention in the chickens for Ca and P retention were outside the range of 46 to 71% observed by Liu *et al*. (2014) though reports were based on the Ca:P ratios in the feed.

The effect of oil types and varying DC levels on Relative primal cuts of Broiler chickens

The live weight was affected ($p<0.05$) by the DC levels, the highest value was recorded at 1.25% DC inclusion than other levels. Thigh weight was highest at 0.75% DC inclusion while other cuts were not affected. The observed increased live weight may be ascribed to the importance of Ca in the nutrition of poultry birds as improved performance has been reported with supplemental Ca in poultry (Bao and Chot, 2007). The reduced relative weights of some of the primal cut could partly be related to poor digestion in the gut microflora which does not allow appropriate release and use of nutrients in the diet. In a study by Atteh and Leeson, (1983) using broilers, they reported a poor relationship between the absorption and retention of Ca and Mg with 8% inclusion of oil. The addition

of free fatty acids in the diet resulted into reduced absorption of divalent minerals. Sklan, (1989) drew his conclusion on the use of monoglycerides in feeds due to their efficient micel formation and fatty acid absorption from the GIT. Atteh and Leeson, (1984) reported that fats with an increased saturated fatty acids reduces mineral absorption and increased mineral excretion with the faeces (alkali salts of fatty acids) than fats containing unsaturated fatty acids. The authors concluded that soluble soaps are obtained from unsaturated fatty acids and could be absorbed efficiently while salts from SFA were unabsorbable and thus excreted.

In pullets, excretion of faecal soap increased when 8% oleic acid was replaced with palmitic acid and Ca retention was reduced by 20% (Atteh and Leeson, 1984). Also, in a review by Allen (1982) using rats, it was observed that saturation level and increasing chain length reduced Ca soap formation. An increase in the Ca concentration could raise the pH of the GIT because the major source of Ca is limestone that has a high acid binding capacity (Jahanian *et al.*, 2008). Results here corroborates that of Shafey and McDonald (1991) that a high intestinal pH reduced absorbable portion of minerals thereby inhibits absorption. The different oil types followed a similar trend with the parameters measured except for broilers on PKO which had the highest wings cut. The present result showed that the DOs had varying fatty acid contents. No credible description can be provided for this observed trend. Increased relative weights of primal cuts with increasing Ca concentration and soybean oil was reported by Shafey and McDonald (1991). Also, Walk *et al*. (2012) reported increased live weight when Ca level was increased from 6.4 to 10.33g/kg in the diets of broiler chickens.

However, the interaction effects of oil type and dietary Ca level of broilers improved wings and breast meat with supplementation of PKO and DC below 1.25% having more influence compared to other dietary combinations. This could be attributed high lauric acid which is in CO was the major fatty acids are lauric was 48%, myristic was 16%. The PKO is a major source of MCFA which of low molecular weight and easily absorbed in the GIT and aids utilisation of nutrients, supplies energy readily and prevent excess fat deposition.

Ketone bodies could be used as a measure of performance and nitrogen balance, because MCFA are ketogenic compared to LCFA (Klein and Miles, 1994) and they increase synthesis of protein in rat and poultry studies. MCFA serve as a good source of energy and lowered the use of protein as energy source. The result obtained here agrees with the findings of Atteh and Lesson, (1984) who included palmitic acid at 80g/kg containing 8, 12, and 16g/kg Ca in broiler diet. They observed high level of excreta soap formation with increase in Ca concentrations. Also, Senkoylun *et al.* (1991) reported that broilers fed diets containing 80g/kg vegetable and animal fat containing 9g/kg Ca had increased insoluble soap than those on 40g/kg fat with 9g/kg dietary Ca.

CHAPTR SIX

SUMMARY AND CONCLUSION

6.1 Summary

Three studies were conducted to assess the influence of different dietary oils on growth performance and calcium retention in broilers. The first experiment was the preliminary study comprising of the selection of different edible and novel oil used in compounding feed for chickens and analysing chemically with the intention of characterising them according to include fatty acid profile, iodine, peroxide, acid and saponification values. The second study involved assessment of the effects varying levels of dietary oils at 1, 2 and 3 % on performance, serum biochemical indices and calcium retention in broilers. The optimum levels of the oil at which best performance of chickens were selected for further studies. The third experiment was aimed at evaluating the effect of the oil types and varying dietary levels of calcium on serum biochemical indices, performance, and calcium retention in broiler chickens.

Physico-chemical characteristics of selected dietary oils

The selected vegetable oils were high in polyunsaturated fatty acid and low in saturated fatty acid. Palm kernel oil is high in polyunsaturated fatty acid with a considerable high saponification value which enhances the shelf life and this property aids absorption and utilization of nutrients.

Coconut oil also has a high saponification value that enhances the absorption and utilization of nutrients in the feed. It is also rich in lauric acid, which is found in mother's milk and a good component in infant formulae which has antimicrobial properties that boost the immune system. Sheabutter is high in α-tocopherol, a powerful antioxidant protecting the cell membrane against harmful free radicals by scavenging or mopping them out. It is also rich in total carotene which is a precursor of provitamin A synthesis in the body.

Effects of varying levels of dietary oils on performance, serum biochemical indices and calcium retention in broiler chickens

The oils had high iodine values, high saponification values with considerable quantities of total carotene and α-tocopherol which increases their ability to form micelles. Thus, serves as good energy supplement, and could ease absorption and digestion of inherent minerals and nutrients in the diets. Dietary oil inclusion at 2% and 1.25% calcium, significantly enhanced calcium retention in broiler chicken. Weight changes and FCR were improved at 2% inclusion of coconut oil in diets of broiler chickens. Serum SOD and GLU activities were enhanced with increasing levels of dietary oils.

Effects of oil types and dietary calcium levels on performance, serum biochemical indices, carcass traits, ileal calcium and phosphorus digestibility in broiler chickens

Tibia calcium retention was enhanced at 0.75% calcium and 2% inclusion of palm kernel oil and coconut oil. Carcass qualities of broilers improved with the combinations of coconut oil and 1.25% calcium levels. At 2% dietary inclusion of the oils, optimum obtained for broiler chickens on PKO and CO with respect to performance indices. Calcium retentions in broiler chickens were improved through the deposition of calcium in the tibia bones with the dietary inclusion of palm kernel oil and coconut oil at 2% and 1.25 dietary calcium.

6.2 Conclusion

Palm kernel oil and coconut oil have low iodine values (4.71, 6.95g/100g) and high saponification values (104.76, 109.81mg/KOH) with total carotene and α-tocopherol. Dietary palm kernel oil and coconut oil at 2% level of inclusion in the diet enhanced calcium retention which prevented excretion of minerals and aided proper utilisation of nutrients in chickens. It also boosted the immune system of the broiler chickens thereby leading to improved growth and performance. Also, the performance of broiler chickens was enhanced and calcium retention improved with dietary oil inclusion at 2% and 1.25% calcium level.

6.3 Recommendation

The inclusion of palm kernel, shea butter and coconut oils in poultry diets needs to be further investigated in laying birds in order to generalise their usage in commercial poultry production.

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