RESPONSE OF BROILER CHICKENS TO SUPPLEMENTAL CHELATED COPPER, ZINC AND MANGANESE OR THEIR CORRESPONDING INORGANIC SOURCES.

BY

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DECLARATION

I hereby declare that this dissertation was written by me and is a correct record of my own research work. It has not been presented in any previous application for any degree of this or any other University. All citations and sources of information are clearly acknowledged by means of references.

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CERTIFICATION

We certify that this dissertation entitled "Response of broiler chickens to supplemental chelated copper, zinc and manganese or their corresponding inorganic sources" is the outcome of the research carried out by A. A. Ayoola in the Livestock Science and Sustainable Environment programme, Centre of Excellence in Agricultural Development and Sustainable Environment (CEADESE), Federal University of Agriculture Abeokuta (FUNAAB).

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ABSTRACT

Trace minerals are essential nutrients that are required for a variety of metabolic functions including immune response, pathogenic challenge, reproduction and growth in broiler chickens. The trial was carried out to evaluate the effect of supplemental Cu, Zn and Mn chelated with 2-hydroxyl-4-methyl thiobutanoic acid (HMTBa), a precursor of DL- methionine, and the inorganic sources on growth performance, trace mineral digestibility, haematology, serum biochemistry and tissue (liver and kidney) trace minerals concentration of broiler chickens. A 42-day feeding trial using a Completely Randomised Design was conducted using a total of three hundred (n=300) Arbor Acre (AA) broiler chickens as experimental birds. The AA broiler chickens were allocated to five dietary treatment groups. Each group had six replicates with ten birds each. The treatments were control group (basal diet), 100% inorganic trace minerals (ITMs) supplemental level (15, 100, 100 mg/kg for Cu, Zn, Mn respectively), 50% ITMs supplemental level (7.5, 50, 50 mg/kg for Cu, Zn, Mn respectively), 50% Chelated trace minerals (CTMs) supplemental level (7.5, 50, 50 mg/kg for Cu, Zn, Mn respectively) and 25% CTM supplemental level (3.75, 25, 25 mg/kg for Cu, Zn, Mn respectively). Data collected were subjected to one – way Analysis of Variance using a Completely Randomised Design. Results showed that the groups fed diet with 100% supplemental levels of ITM and 50% supplemental level of CTM improved ($p<0.05$) feed conversion ratio of 1.55 and 1.54 respectively between 0 and 21 days of the experiment. The group fed 50% supplemental level of CTM had a reduced ($p<0.05$) daily feed intake of 39.79 g than the group fed 100% supplemental ITM level (41.91 g). Daily feed intake and daily weight gain were higher ($p<0.05$) for the birds fed 100% supplemental ITM level (146.29 g and 73.54 g) and 50% supplemental CTM level (140.18 g and 73.53 g respectively) between 21 – 42 days of the experiment. A lower ($p<0.05$) white blood cell count ($11.07 \times 10^6/mm^3$) and an improved ($p<0.05$) red blood cell count ($13 \times 10^6/mm^3$) was observed for the group fed 50% CTM supplemental level. The group fed 50% supplemental diet of CTM had the highest ($p<0.05$) total protein (73.2 g/L), albumin (41.20 g/L) and globulin (32 g/L) in serum as compared to the other groups. The serum Cu (16.5 ug/dL), Zn (14.13 ug/dL) and Mn (1.88 ug/dl) were significantly higher ($p<0.05$) for the group fed 25% supplemental diet of CTM. The group fed 25% supplemental diet of CTM had a higher ($p<0.05$) High Density Lipid (HDL) of 50.87 mg/dL. The liver-Zn (4.22 mg/100 g) and Liver-Mn (0.50 mg/100 g) and kidney-Cu (1.17 mg/100 g) for the group fed 25% supplemental diet of CTM were the highest ($p<0.05$) compared to the other groups fed supplemental diets of trace minerals. The Zn level of 7.12 mg/100g in kidney for the group fed 100% ITM supplemental level was lower ($p<0.05$) than the groups fed supplemental diet of CTM. The study concluded that trace minerals supplementation improved growth performance of broiler chickens while the chelated form improved the bioavailability of the trace minerals and reduced minerals excreta into the environment.

DEDICATION

I dedicate this dissertation to God Almighty for His Grace and Mercy to finish this programme and also to my lovely wife, Barr. O. B. Ayoola for her priceless support and invaluable encouragement that gear me up to focus and complete this programme.

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CHAPTER ONE

1.0 INTRODUCTION

Trace minerals are essential nutrients in broiler chicken's diets and they play very important roles in birds and these include cell proliferation and growth, tissue and bone development and integrity, immune development and response, reproduction, enzymes formation, gene regulation, and protection against oxidative stress and damage (Shankar and Prasad, 1998; Underwood and Suttle, 1999; Fraker *et al*., 2000; Blanchard *et al*., 2001; Ibs and Rink, 2003; Song *et al*., 2009; Richards *et al*., 2010). Trace minerals can only be required in a diet at a lower inclusion level because of the increase in the environmental pollution from the large scale poultry industry which has been linked with the excretion of the un-utilised minerals (Paik, 2000; Koutsos, 2011).

Copper (Cu), zinc (Zn) and manganese (Mn) are biologically essential trace minerals needed in broilers' diet (Richards *et al*., 2010) and studies have shown that these trace minerals have potential roles in boosting cellular and humoral immunity in broilers (Kidd *et al*., 1992; Kidd *et al*., 1993). Inorganic trace minerals (ITM) such as sulfates of Cu, oxides of Zn and Mn are often being used in broiler chicken diets at the higher inclusion level of five times more than the recommended quantity of 8, 40 and 60 mg/kg for Cu, Zn and Mn respectively by NRC (1994), but the absorption is limited, because of the antagonistic effect in diet and gastrointestinal tract (Richards *et al*., 2010; Manangi *et al*., 2012). Naturally, ITMs are used to meet the mineral requirements of the broiler chickens (Das *et al*., 2014) and feed producers tend to add ITM more than what is required or recommended (Lee *et al*., 2001; Paik, 2001; Manangi *et al*., 2012) and this results to increasing concerns over the excretion of minerals as pollutant in the environment (Manangi *et al*., 2012).

A chelated mineral means the bonding or combination of metal ions with organic ligand or ligand complex such as amino acids, proteinate, polysaccharides or organic yeast (AAFCO, 1998; Dieck., *et al*., 2003; Bao, *et al*., 2006). In addition, chelated minerals are minerals that are protected from other minerals interaction when bounded with chelating agents like amino acids or proteins (Pal and Gowda, 2015). These chelated minerals are protected by chelating agents from other minerals interference in the gastrointestinal tract and more stable and less reactive in the digestive tract (Pal and Gowda, 2015). The supplementations of these chelated minerals are better in terms of absorption than other forms of mineral sources (Yi *et al*., 2007).

The chemical form of trace mineral sources significantly influences the absorption and bioavailability of the minerals (Das *et al*., 2014). Bioavailability of trace minerals may vary from as low as 5-20% and as high as 80-90% for inorganic trace minerals depending on physical forms and reactions with other dietary nutrients (Cano-Sancho *et al*., 2014). On the other hand, amino acids are ideal ligand for minerals to create a more stable and bioavailable form of trace minerals (Yi *et al*., 2007). A functional amino acid chelate is electrically neutral, it has a constant stability and contains easily metabolized ligand and, this is however assumed to be more bioavailable (Seo *et al*., 2008) to the broilers.

Mateos *et al*., (2005) also reported that CTMs have more benefits to the birds and these include: protection from unwanted chemical reactions in gastrointestinal tract; ensure smooth passage through intestine wall without any chemical interference by other minerals; and absolute absorption through different digestives routes. A report also showed that CTMs have greater bioavailability in broilers (Kidd *et al*., 2000; Abdallah *et al*., 2009; Ao *et al*., 2009) and are better absorbed and utilized than their inorganic counterparts and are protected from other mineral interactions that interfere with their bioavailability that could lead to a reduction in the excretion of minerals (Scott *et al*., 1982; Paik, 2001; Leeson, 2003) to the environment. Lee *et al*., (2001) reported that CTMs as a supplement for broilers can be used in a conventional diets, but when it exceeds the bird's requirement, a negative digestibility of trace minerals will occur (Paik, 2001; Pal, *et al*., 2010).

1.1 JUSTIFICATION

Trace minerals, such as Zn, Cu, and Mn, are required to ensure good health and optimum performance of the animal (Manangi *et al*., 2012). They function as enzyme cofactors and are also constituents of metalloenzymes. Zinc plays a prominent role in the synthesis of collagen and keratin (Underwood and Suttle 2001). Collagen is an important structural protein of essential tissues, such as cartilage and bone, while keratin is the structural protein of the feathers, skin, beak, and claws. Copper plays a vital role in the proper cross-linking of collagen and elastin (McDowell 2003), and Mn is essential for the maintenance of bone mineralization (Manangi *et al*., 2012). Both organic and inorganic forms of these minerals exist.

Studies have shown that the absorption of ITMs is sometimes restricted as a result of mineral antagonisms in both diet and gastrointestinal tract (Yi *et al*., 2007; Richards *et al*., 2010; Echeverry *et al*., 2016) of broiler chicken. Nigeria broilers' production industries are heavily depended on the use of ITM in broiler diets as a result of high cost and the inadequate availability of CTM. Chelated trace minerals has been reported to have enormous benefits of improving performance of the birds and also sustaining the environment (Paik, 2000, Pal, *et al*., 2010). Chelated trace minerals have several benefits when supplemented in broilers' diets and these include protecting the essential trace minerals from other minerals that are highly chemically reactive in gastrointestinal tract and ensuring absolute absorption without any interference by other minerals (Li, *et al*., 2004; Mateos, *et al*., 2005; Abdallah *et al*., 2009; Ao *et al*., 2009 Koutsos, 2011).

Furthermore, much of the information even in recent NRC documents is actually based on research from 1960s and 1970s when the management and genetic modification of these birds were different (Leeson 2005; Nollet *et al*., 2008). As a result of this, the NRC recommendation of trace minerals may not be a true representation of the current day requirement for broiler chickens.

This study aims at evaluating the response of broiler chicken fed diets supplemented with inorganic trace minerals (ITM) and chelated trace minerals (CTM).

1.2 BROAD OBJECTIVE:

To investigate the response of broiler chickens to supplemental chelated trace minerals (Zn, Cu and Mn)

1.3 SPECIFIC OBJECTIVES:

1. To determine the effect of ITMs and CTMs supplementation in the diets of broiler chickens on growth performance (daily feed intake, average body weight, daily weight gain, feed conversion ratio, livability and lameness)
2. To determine the effect of ITMs and CTMs supplementation on nutrients digestibility of broiler chickens.
3. To determine heamatological indices and trace mineral concentration in serum of broiler chickens fed diet supplemented with ITMs and CTMs (Cu, Zn, Mn).
4. To determine the concentration of selected minerals (Cu, Zn, Mn) in tissues (kidney and liver) of broiler chickens fed diets supplemented with ITMs and CTMs

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Poultry production

Over recent decades the poultry production industries have made several improved changes in order to meet the increasing demand for cheap and safe supply of meat and eggs (FAO, 2006a; Paik, 2001). In three decades as well, the poultry industries have also been experiencing a significant growth of 5 percent or more per annum (against that of 3 percent for pig meat and 1.5 percent for bovine meat) and its contribution in world meat production also increased from 15 - 30 percent in three decades (FAO, 2006a).

The growth of the poultry industry has also been accompanied by structural changes within the sector, characterized by the emergence and growth of "land-independent" (industrial) farming establishments, and the intensification and concentration of poultry operations (FAO, 2006a). Pressure to lower production costs and increase supply has led to more efficient operations, made possible through the shift to larger, specialized and more integrated facilities, and through the improvements in the use of animal genetics, advancement in nutrition and current production techniques (FAO, 2007; Thornton and Gerber, 2010). The driving forces behind structural change in poultry production are no different than those that affect other livestock commodities: market pull, innovation and economies of scale. Economies size and innovation that contributed to the livestock sectors have also served to distinguish animal production from crop production. Several large and specialized sectors today focus on producing animals, and purchase most of their feed. This often means that there is limited access to land on which to spread manure (Paik, 2001).

2.2 Broiler production

Broiler chickens (*Gallus gallus domesticus*) are a gallinaceous (an order of heavy-bodied ground feeding birds) domesticated fowl, bred and raised specifically for meat purposes (Kruchten, 2002). They grow much faster than egg laying hens or dual purpose breeds, and most broilers have a fast growth rate with a high feed conversion ratio and low activity levels (Garrigus, 2007). In five weeks, broilers can reach a dressed weight of 1.8 – 2.27kg (4-5 pounds) in association with adequate feeding and management methods to support the growth while the dual-purpose breeds are usually raised for both meat and egg production, and are smaller with a slower growth rate (Bressei, 2006).

2.3 Definition of minerals

Minerals are inorganic elements, present in all body tissues and fluids. Although minerals yield no energy, their presence is necessary for the maintenance of wide variety of physiological processes which are essential to life (Soetan, 2010). Twenty two minerals are believed to be essential for bird life: seven macro-elements (calcium, phosphorus, potassium, sodium, chlorine, magnesium a d sulfur) and 15 trace minerals (zinc, manganese, copper, iron, selenium, iodine, cobalt, molybdenum, chromium, tin, vanadium, fluorine, silicon, nickel and arsenic) (Suttle, 2010). The macro-elements usually stated as a percentage, while trace minerals are presented in mg/kg of the diet. This classification does not reflect importance of these nutrients, but only necessary to quantify them in diets, or their concentration in bird tissues (Lukic *et al.*, 2009).

2.3.1 Minerals in animal nutrition

All animal and plant tissues contain widely varying amounts and proportions of mineral elements, which largely remain as oxides, carbonates, phosphates and sulfates in the ash after ignition of organic matter (Suttle, 2010). These minerals are inorganic substances that are found in all body tissues and fluids and their presence in animal body is essential for the maintenance of certain physiochemical processes and chemical constituents are used by the body in several ways but it does not produce energy (Malhotra, 1998; Eruvbetine, 2003). All animal tissues contain 20–30 mineral elements, mostly in small and variable concentrations and the level of requirement does not reflect the degree of importance but the necessary quantities in diets or their generally low concentration in tissues (Lukic *et al*., 2009). These are probably adventitious constituents, arising from contact with a chemically diverse environment (Suzuki *et al*., 2005).

2.3.2 Importance of mineral in nutrition of poultry

Trace mineral nutrition in poultry is a complex area that involves an extra care due to the large number of minerals and dietary inclusion levels as well as potential interactions between the minerals (Koutsos, 2011; Lopez-Alonso, 2012). Macro - minerals are needed by the birds in quantities great enough to be expressed as a significant percentage of the diet and these include calcium, phosphorus, sodium, potassium, magnesium and chloride while micro – minerals or trace elements are minerals required in smaller amounts but essential in birds' diets and these include copper, zinc, manganese, iodine, iron and selenium (Koutsos, 2011). Copper, zinc and manganese are metal cations with several essential functions in the body. The major role of copper, zinc and manganese is a structural or catalytical component of enzymes. (Cater and Mercer, 2005).

2.3.3 Macro minerals

Macro-minerals are required in large quantities and include calcium, chloride, magnesium, phosphorous, potassium, sodium and sulphur (Koutsos, 2011). These minerals required in a large amount are needed in amount higher than 100mg/day (Murray *et al*., 2000; Mahan and Escott-Stump, 2005). They are part of structural components of tissues, act as electrolytes in fluids, and serve as catalysts in the endocrine system or in enzymes (McDowell, 2003; Cater and Mercer, 2005). An inadequate supply of macro minerals and trace elements will lead to a deficiency and cause biochemical dysfunction, disturbed physiological functions or structural disorders (McDowell, 2003; Schweinzer *et al*., 2017). For example, macro minerals such as K are important for osmotic pressure and normal acid-base balance, Mg is involved in enzymatic reactions and is required for normal operation of the nervous system, and Ca is mostly found in skeletal tissues and is essential for muscle contraction (Sykes, 2007; Rankins and Pugh, 2012). Overfeeding macro minerals could result in higher mineral excretion in manure and can lead to an excessive salt load in soils after multiple years of manure application (Chang *et al*., 2004).

2.3.3.1 Calcium

Calcium is an important macro mineral and one of the most abundant elements in the earth's crust as well as the most abundant cation in the animal body (Hemati-Matin, 2013). Calcium is important to skeletal structure and strength because the primary mineral form in bone is hydroxyapatite ($Ca_{10} (PO_4)_6(OH)_2$) (Palmer, *et al*., 2008). Calcium is retained mostly in bone and ninety-nine percent of Ca is located in bones and teeth (Underwood and Suttle, 1999). Calcium is involved in a large number of enzymes such as adenosine triphosphatase (ATPase), succinic dehydrogenase, lipase etc, and also required for membrane permeability, muscle contraction, normal transmission of nerve impulses and in neuromuscular excitability (Malhotra, 1998; Murray *et al*., 2000). Bone Ca to P ratio is approximately 2:1 (Underwood and Suttle. 1999). Calcium also serves in nerve impulses, secondary messengers in cell signaling, enzyme activation, muscle control, and is associated with blood clotting (Lodish, *et al*., 2000).

Calcium in the diet can affect the metabolism of several other minerals. Low Ca in the diet may have negative effects on serum P (Hsu *et al*., 1975), however, a high Ca to P ratio also has a detrimental effect of P absorption. High Ca levels are also antagonistic on Mg absorption (O‟Dell, 1989).

Calcium is absorbed into the cells by both active and passive transport and much of it is vitamin D dependent (Bronner, 1998). Calcium homeostasis is controlled by absorption, urinary excretion, and bone turnover (Fairweather-Tait and Hurrel, 1996).

2.3.3.2 Phosphorus

Phosphorus is found in every cell of the body and often concerned with many metabolic processes such as buffer in body fluids and retained mostly in bone and soft tissues (e.g. muscle) (Hays and Swenson, 1985). Bone Ca to P ratio is approximately 2:1 (Underwood and Suttle. 1999). Phosphorus is also important to skeletal structure and strength because the primary mineral form in bone is hydroxyapatite ($Ca_{10} (PO_4)_6(OH)_2$) (Palmer, *et al*., 2008). Eighty percent of Phosphorus is located in bones and teeth and it is the second largest constituents in bones and teeth (Underwood and Suttle, 1999).

Phosphorus is part of nucleic acids, phospholipids in cell membranes, and energy transfer (ATP and GTP) (Underwood and Suttle, 1999). Phosphate also plays a role in osmotic and acid-base balance as HPO_4^{2-} and $H_2PO_4^-$ (Wood, 2006). Molecules are also phosphorylated to trap them in a cell or to activate/inactivate enzymes.

Intestinal P is absorbed by the Na-dependent brush border transporter and by non saturable processes (Wood, 2006). Circulating 1, $25(OH)_2 D_3$ also increases P absorption. Deficiency of phosphorus in poultry could result to symptoms cause rickets and osteomalacia and bone loss (Malhotra, 1998; Murray *et al*., 2000).

2.3.3.3 Magnesium

Potassium and Magnesium are the primary minerals found in the intracellular fluid, most K is in muscle, and most Mg is in bone (Underwood and Suttle, 1999). Magnesium is a constituent of many enzymes (such as myokinase, diphosphopyridinenucleotide kinase, creatine kinase etc) and of bone (Murray *et al*., 2000). It is also the second most abundant intracellular mineral found in bone and also aids the carbohydrate and fat metabolisms, help in muscle contraction, maintain proper blood calcium levels and acts as catalysts for a wide range of enzymes (Underwood and Suttle, 1999; Ilich, 2000). But the primary role of Mg inside the cell is the neutralization of negatively charged molecules such as ATP, ADP, DNA, and RNA; Mg associates with negatively charged molecules so that together they have no overall charge (Konrad and Schlingmann, 2006). Through its association with ATP, many energy-requiring processes are reduced by Mg deficiency. Magnesium is also the cofactor of enolase and pyruvate kinase, both of which are enzymes in glycolysis (Berg, *et al*., 2002).

Magnesium is absorbed in the small intestine by both diffusion and saturable processes (Fine *et al*., 1991). The saturable process is more evident in periods of low Mg intake (Coudray *et al*., 2003). Circulating Mg is either bound to protein or ionic. Magnesium is stored in bone or excreted in urine (Fairweather-Tait and Hurrel, 1996; Underwood and Suttle, 1999). Instead of being free ions, much of the intercellular Mg ions are complexed to negatively charged molecules including ATP (Konrad and Schlingmann, 2006).

2.3.4 Micro minerals/Trace minerals

Micro minerals are minerals that are required in smaller amounts for normal functioning of the animal and they include copper, zinc, manganese, iodine, iron and selenium. Trace minerals are involved in the animals’ metabolism as catalytic agents present in all metabolic reactions, and therefore, they are essential for growth, development, and production (Aksu *et al*., 2012). Often times, trace minerals are being neglected, compromised with or underestimated by the producers while macronutrients such as energy, protein and macro minerals are given major attention when nutrients requirement of poultry is being formulated (Jegede *et al*., 2011). However, trace minerals, such as Cu, Fe, Zn, and Mn are essential for broiler growth and are involved in many digestive, physiological, and biosynthetic processes within the body. They function primarily as catalysts in enzyme systems within cells or as coenzyme factors. They are also constituents of hundreds of proteins involved in intermediary metabolism, hormone secretion pathways, and immune defense systems (Milanović *et al*., 2008; Sunder *et al*., 2008).

Deficiency symptoms are typically manifested as disturbances in multiple metabolic processes, resulting in lower production performance, loss of appetite, reproductive disorders, and impaired immune response (Van Der Klis and Kemme, 2002). This can be caused by inadequate mineral intake or by the presence of antagonists in the diet or complexity of the large number of minerals and dietary inclusion levels (Koutsos, 2011)

2.3.5 Biological functions of trace minerals

The dietary minerals are usually transported from the serosal side of the mucosa to the liver in free or bound forms via the portal bloodstream (Suttle, 2010). From the liver, they are transported by the peripheral bloodstream to be taken up by different organs and tissues at rates determined by local transporter mechanisms in cell membranes and organelles to meet intracellular needs. Minerals perform four major types of biological functions: Structural: minerals can form structural components of body organs and tissues. Physiological: minerals occur in body fluids and tissues as electrolytes concerned with the maintenance of osmotic pressure, acid-base balance, membrane permeability and transmission of nerve impulses. Catalytic: minerals can act as catalysts in enzyme and endocrine systems, as integral and specific components of the structure of metalloenzymes and hormones or as activators (coenzymes) within those systems. Regulatory: minerals regulate cell replication and differentiation (Suttle, 2010).

The macro-elements are playing structural roles, while trace minerals primarily act as catalysts in enzyme systems within cells or as parts of enzymes and as physiological regulators (Bao *et al*., 2010). Deficiency of trace minerals will definitely affect broiler growth, while surplus trace minerals will not benefit bird growth and will possibly be excreted (Bao *et al*., 2010).

2.3.6 Trace minerals toxicity in birds

All minerals can cause toxicosis in animals, when consumed in large quantities (NRC, 2005). The margin of safety between the minimum amount required to animal feed and the amount that causes adverse effects in animal health varies for different minerals (Bampidis, *et al*., 2013). However, it was also cited by Bampidis, *et al*., (2013) that many minerals that do not contribute in any known functions of the animal body weight and, in fact, are harmful-toxic. Many heavy metals are used as a trace elements and feed additives in poultry feed. These metals are common in our environment, some of these (iron, copper, manganese, zinc, etc) are essential for good health, however; other (Arsenic, mercury, lead, cadmium, etc) are poisonous and deleterious for health (Jadhav *et al*., 2007).

2.3.7 Trace mineral requirements of broiler

Due to the highly reactive nature, the gross excess of copper, zinc and manganese can cause significant pathological effect in broilers. Trace mineral requirements in broilers are variable and depend on age (Devi *et al*., 2011) sex (Yatoo *et al*., 2012), stage of growth or production (NRC, 1996; NRC, 2001) breed and genotype (Lukić *et al*., 2009; NRC, 1994; Waldroup, 2001). They are recommended by different agencies like National Research Council, India (NRC, 1996; NRC, 2001; NRC, 1994). Agricultural Research Council, India (ARC, 1980) or researchers (Chiba, 2009; Suttle, 2010) in both poultry and other livestock.

Micro – minerals are those required in smaller amounts and include copper, zinc, manganese, iodine, iron and selenium. Copper, zinc and manganese are metal cations with numerous essential functions in the body. The predominant role of copper, zinc and manganese is as structural or catalytical components of enzymes (Scheideler, 2008).

2.3.8 Copper

Copper is one of the essential trace minerals and an important mineral in poultry nutrition. It contributes to bone development and most especially cartilage formation. Copper is involved in blood proteins, iron metabolism and absorption, oxygen metabolism, collagen and elastin synthesis, bone formation, feather development and coloring (Chandra, 1990; Uany *et al*., 1998; Scheideler, 2008). Leeson and Summers, (2001) reported that copper is needed by most animal to prevent microcytic hypochromic anaemia which has a great effect on iron metabolism. It also plays various roles in enzyme systems such as cytochrome oxidase, amino oxidase and polyphenoloxidase (Kim and Hill, 1996; Leeson and Summers, 2001). Copper is potentially toxic to birds although avian toxicity from this metal is less common (Miles *et al*., 1998; Luo, 2005). Acidic foods stored in copper containers may leach out copper, and occasionally copper piping for water is a potential source of increased copper in the diet if the water is slightly acidic and has been allowed to remain in contact with the piping for some length of time. Allowing the water from the tap to run for a few minutes before filling the water dishes will prevent this problem.

Copper is also essential for energy production (cytochrome oxidase), connective tissue formation (lysyl oxidase), antioxidant defense (copper/zinc superoxide dismutase), pigment formation (tyrosinase), iron transport and metabolism (ceruloplasmin) and hormone and neurotransmitter synthesis (dopamine beta-monooxygenase) (Cater and Mercer, 2005).

2.3.8.1 Metabolism of copper

The dietary sources of copper can determine the biological availability of copper to birds for absorption and utilization depending on whether the sources are inorganic (such as copper sulphate, copper oxide etc) or chelated sources (such as complexes or proteinates) of copper (Igbasan and Akinsanmi, 2012). Requirement of copper values average 6-8 ppm which is traditionally supplied by inorganic salts and most especially copper sulphate, and other inorganic sources vary from 40-115% bioavailability of Cu compared to sulphate (Leeson, 2009). Copper is easily complexed with amino acids or proteins, leading to the development of so-called chelated sources of copper which are claimed to have better digestibility and/or less formation of insoluble complexes with other minerals in the digesta (Pal et al., 2010). Studies have shown that the effectiveness of chelated sources against inorganic forms of Cu is variable, although a research has shown the potential of chelated copper to use much lower levels of supplementation (Zhao *et al*., 2014). Since at least 80% of dietary copper appears in the excreta, using lower levels of diet supplementation means reduced Cu in the environment. With less supplementation, knowledge about bioavailability of Cu in major feed ingredients becomes important (Zhao *et al*., 2014). Copper in cereals is reported to be 80% available to the bird while that in vegetable protein is closer to 50% available. Availability from animal proteins is variable, while corn distiller's grains now provide the most concentrated source of Cu within the major ingredients (Leeson, 2009). Copper levels greatly in excess of requirement, at around 125 ppm, have been shown to improve performance of meat birds and egg layers. The mode of action is unknown although likely relates to antibacterial properties of Cu. Likewise high levels of diet Cu have been shown to reduce cholesterol content of eggs and poultry meat, although this is often at the expense of loss in performance and contribution of more Cu to the environment (Leeson, 2009).

2.3.8.2 Copper deficiency and toxicity

Deficiency of copper sources can lead to any physiological issues and variety of diseases (Iqbal *et al*., 2012). Copper deficiency in poultry is normally associated with bone abnormalities, impaired immune responses and anemia (Savage *et al*., 1966).

Copper toxicity is less of a concern in poultry than in other species such as sheep, although toxicity symptoms like reductions in feed intake and bodyweight gain can be seen when feeding extremely high amounts of copper, i.e., greater than 500 parts per million (Miles *et al*., 1998; Luo, 2005).

2.3.8.3 Bioavailability of different copper sources

There are several sources of copper, zinc and manganese, including animal and plant products, mineral salts (such as sulfates, oxides etc), chelated or complexed minerals (such as complexes with amino acids. Proteinate or polysaccharides) and hydroxy minerals, e.g., tribasic copper chloride (TBCC) (Littell *et al*., 1997;Yi *et al*., 2007; Lu *et al*., 2010; Wang *et al*., 2010). The latter two categories represent relatively new technologies in the production of minerals that offer several advantages over traditional mineral sulfates and oxides (Littell *et al*., 1997; Lu *et al*., 2010). It is notable that the chelated mineral category includes a number of potential substrates for mineral binding, including amino acids, peptides and polysaccharides, and there are differences in quality between chelated or complexed mineral sources (Lu *et al*., 2010). High-quality chelated minerals and hydroxyl minerals offer advantages over traditional mineral sulfates and oxides for several reasons, including higher relative bioavailability, lower risk of toxicity, higher stability in feed and greater bio-efficacy (Lu *et al*., 2010; Koutsos, 2011). First, hydroxyl and high-quality chelated minerals generally have higher relative bioavailability (RBV) compared to traditional mineral sources (Koutsos, 2011). Improved RBV means that more of the nutrient is absorbed in a form that can be used by the animal (Littell *et al*., 1997). Oxide-bound mineral is very strong covalent bond and this will prevent dissociation in the gastrointestinal tract and, invariably will reduce RBV (Koutsos, 2011). Moderately covalent-bound minerals like hydroxyl and chelated minerals will have more stability in feed and the gastrointestinal tract but, due to solubility in the gastrointestinal environment, will be available at appropriate locations for mineral absorption (Linder, 1991; Guo *et al*., 2001;Arias and Koutsos, 2006)). For example, when broiler chicks were fed ionically bound copper sulfate compared to covalently bound TBCC, more copper was unavailable (not-extractable) when copper sulfate was fed (Naziripour and Klasing, 2010). This shows that copper from TBCC would be more available for absorption throughout the gastrointestinal tract as against the whole absorption at the upper gastrointestinal tract (Luo et al., 2005; Arias and Koutsos, 2006; Lu et al., 2010). The consequences of improved RBV are that when animals are fed at the nutritional requirement, less mineral needs to be incorporated into the diet than when using mineral sources with lower RBV and this regime of feeding strategy can be adopted when considering the environmental impact of overfeeding minerals (Koutsos, 2011).

2.3.9 Zinc

Zinc is an essential trace minerals and it plays several roles in structural and catalytic functions in proteins, with enzymes involved in bone synthesis, resorption and remodeling (Vallee *et al*., 1991; Ford, 2004;), the protein metallothionein that plays a role in intracellular metal metabolism and storage (Davis and Cousins, 2000; Cousins *et al*., 2006; Koustsos, 2011) and as a modulator of immune function. Zinc modulates the inflammatory response (Peterson *et al.,* 2008), development of immune organs and functionality of immune cells (Rink and Haase, 2007).

Zinc as a co-factor for many enzymes, is also associated with classes of enzymes such as alchol dehydrogenases, carboxy peptidase, alkaline phosphatase, thymidine kinase, DNA polymerase and RNA and variety of transcription factors (Vallee and Falchuk, 1993; Underwood and Suttle, 1999).

Zinc also plays a wide range of essential roles in broilers fed diets supplemented with Zn sources and these roles include cell proliferation, better growth rate and protection against oxidative stress and damage (Shankar and Prasad, 1998; Underwood and Suttle, 1999; Fraker *et al*., 2000; Blanchard *et al*., 2001; Ibs and Rink, 2003; Song *et al*., 2009). The roles of zinc in gene regulation are also crucial when incorporated in the structure of various transcription factors such as hormone receptor proteins (Coleman, 1992; Blanchard *et al*., 2001, Dreosti, 2001; Cousins *et al*., 2003) and as well as synthesis of variety of enzymes and other structural proteins such as collagen and keratin that both required zinc for their synthesis (Underwood ans Suttle, 1999).

2.3.9.1 Zinc metabolism

A major goal of many poultry producers is to attain good flock liveability (kidd *et al*., 1994). Historically, most poultry producers have manipulated environmental conditions and management to maximize bird health (Rink and Haase, 2007). In the past two decades there has been much research into nutritional regimes that improve bird health through immunomodulation (kidd *et al*., 1994). Commercial poultry environments are heavily surrounded with micro-organisms that pose a continuous challenge of the birds' immune system (Kidd *et al*., 2000; Gajula *et al*., 2011). Nutritional supplements that contribute to immune system function may improve flock performance and be economically advantageous (Gajula *et al*., 2011). Dietary zinc (inorganic and chelated or zinc AA complex forms) is essential for normal intestinal barrier function and regeneration of intestinal epithelium (Kidd et al., 2000; Koutsos, 2011). When birds are exposed to heat stress (HS), this can affect intestinal integrity negatively and energy intake and a possible nutritional correction strategy will be needed to improve health, performance, and physical activity (Kidd *et al*., 1994) of the birds. Stefanidou *et al*. (2006) reported that Zn is one of the most important trace elements and plays three major biological roles in the body: as a structural, catalyst or regulatory ion. Zinc is a required co-factor for several hundred enzymes.

2.3.9.2 Bioavailability of different zinc sources

A report indicated that inorganic trace mineral compounds (bioplexes) have a higher value than inorganic forms, the use of bioplexes (chelated mineral complex) as a source of micro minerals complex for livestock has increased in recent years (Switkiewicz and Koreleski, 1998; Switkiewicz *et al.,* 2000).

It was cited by Switkiewicz *et al.,* (2000), that the bioavailability of zinc from a chelated complex with amino acids (ZnAA) was higher than the inorganic zinc sulphate (ZnS04) supplemented in a broiler chicken diets. The use of chelated ZnAA as Zn source had a positive effect on broiler performance, Zn balance, and as well as increased the content of this micro element in the tibia (Koutsos, 2011).

2.3.9.3 Zinc deficiency and toxicity

Zinc is an essential mineral and the first limiting trace minerals among the other three trace minerals such as Cu, Fe and Mn, and zinc deficiency has a major effect on the overall birds' performance even when other minerals are adequately supplemented in diets (Bao et al., 2010). Deficiencies in zinc can cause decreased antibody responses to vaccination (Rink and Haase, 2007; Reed *et al.*, 2015). A research has shown that with inorganic zinc sources (such as sulfate or oxide) and chelated zinc sources (zinc methionine and HMTBa-chelated zinc) in poultry diets have demonstrated several differences in both immune development and response to antigenic challenge (Dibner, 2005; Moghaddam and Jahanian, 2009). Zinc deficiency has no effect on organic Fe uptake by tibia bone but lower the total Fe content to be absorbed (Bao *et al*., 2010). Zinc deficiency, however results in reductions in feed intake, bodyweight gain, hatchability and feather growth (Leeson and Summers, 2001), as well as pancreatic insufficiency (Keinholz *et al*., 1975; McCormick, 1984; Bao *et al*., 2010). Keratin and collagen are major structural proteins, the deficiency of Zn in broilers can reduce the keratin and collagen synthesis rates and this will lead to variety of defects such as bone deformities, poor feathering, reduce tissue strength and skin problems (Underwood and Suttle, 1999; Leeson and Summers, 2001)

Zinc can be extremely toxic to birds when large amount is consumed. Sources of zinc include galvanized cage wire, transport cages, zippers, chrome, zinc hardware, water and feed trough and some antirust paints, (Lu *et al*., 1990; Senthikumar *et al*., 2016).

When zinc is consumed in excess by birds, the clinical signs are lethargy, shallow respiration, anorexia / reduced appetite, decreased body weight, weakness; falling of perch; unable to walk, stand straight, diarrhea, hemolytic anemia, kidney dysfunction, possible liver and pancreatic abnormalities, feather pickings, pale mucous membranes and death (Senthikumar *et al*., 2016). Zinc toxicity (greater than 500 ppm) can also irritate the gastrointestinal tract, cause reduced absorption of other nutrients and systemically will disrupt the functions of proteins, enzymes and DNA (Lu *et al*., 1990; NRC, 2005).

2.3.10 Manganese

Manganese (Mn) is an essential trace mineral of poultry nutrition and it involves in several roles as activator of many enzymes involved in lipids and carbohydrates metabolism (Olgun, 2017). Manganese helps as an antioxidant protection (manganese-super oxide dismutase; de Rosa *et al*., 1980) and as well as cofactor for several enzymes (Au *et al*., 2008). Manganese functions in bone metabolism, just like the case zinc and copper in bone, (Beattie and Avenall, 1992), and it also plays important roles in bone development, eggshell quality, growth rate, fertility, maintenance of performance and perosis prevention in poultry (Underwood and Suttle, 1999; Olgun, 2017).

2.3.10.1 Manganese metabolism

Manganese (Mn) is an element required in nutrition, functioning largely in the enzyme systems involved in lipid and carbohydrate metabolism (Li *et al*., 2011;Olgun, 2017). It plays an important role in growth, bone development, perosis prevention, optimal eggshell quality and performance maintenance of poultry (Underwood and Suttle, 1999; Olgun, 2017). The current NRC (1994) guidelines recommend 60 mg/kg of Mn to be supplemented in broilers diet and 20mg/kg for laying hens. A researcher has suggested that the dietary requirement of Mn may be considered higher (120 – 130ppm) than the (NRC, 1994) values (Li *et al*., 2010) and this helps to enhance heart manganese – super oxide dismutase messenger RNA level (Li *et al*., 2011) and also prevent hypersensitive response (Gajula *et al*., 2011). Another studies cited by Olgun, (2017) suggested that the dietary Mn requirement of laying hens and broilers may be as high as 90 mg/kg, and the Mn-sulphate availability is often higher than that of other inorganic Mn sources but lower than its chelated sources.

2.3.10.2 Manganese deficiency and toxicity

Manganese, an important activator of several enzymes will create several health challenges when deficient in diets and its deficiency will result to a reduction in activity of manganese-super oxide dismutase (Li, *et al*., 2010), induces perosis or lameness (Luo, *et al*., 2007) and increases abdominal fat deposition (Lu, 2007).

Manganese should be supplemented at an optimal requirement level but when a large amount of manganese (greater than 3,000 ppm) is consumed, the following will occur; reduction in feed intake in chicks (Black *et al*., 1985) and this will lead to progressive neurological deterioration and impaired hemoglobin formation (Finley and Davis, 1999; Koutsos, 2011).

2.4 Chelated trace minerals

The term "Chelated mineral" refers to a variety of compounds including metal - amino acid complexes, metal., amino chelates, metal proteinates, metal-polysaccharide complexes, metal-yeast complexes, and metal-organic acid complexes (Patton, 1990; Wang *et al*., 2010; Pal *et al*., 2010). An chelated mineral is simply a combination of a metal ion with an organic ligand such as amino acids, proteins, polysaccharides, yeast, or organic acids (Arias and Koutsos, 2006; Pal *et al*., 2010).

Specifically, the metal ion is bound to a ligand through multiple attachments (either ionic or covalent) with the metal ion occupying a central position in the structure (Kincaid, 1989, Nelson, 1988; Pal and Gowda, 2015). When chelated mineral formation occurs, the metal ion and organic ligand act as mutual electron donors (ligand) and electron acceptors (metal cations) producing a heterocyclic ring structure (Nelson, 1988). Generally, the metal ion is attached to electronegative areas (two or more) on a ligand. Chelated minerals can be classified into two categories: natural and synthetic.

Natural mineral complexes are formed during normal digestion, absorption, and metabolism in a living system. Synthetic mineral complexes (usually by dietary supplementation) conversely, are used to enhance mineral utilization efficiency. During digestion, a variety of natural mineral complexes are formed which either enhance or diminish the usefulness of the ingested minerals.

Herrick (1993) categorized natural minerals into three types based on their function in biological systems. These include complexes which: transport and store metal ions, are essential to physiological activity, and interfere with metal ion utilization. Amino acids, EDTA, and other synthetic ligands are important as metal binding and transporting agents in the gastrointestinal tract, which enhance uptake of metal ions from the intestinal lumen into the mucosal cells. For instance, transferrin is essential for gut absorption, transport, and storage of iron. Additionally, metal complexes may form in biological systems to allow physiological activity of certain compounds. Hemoglobin contains iron and vitamin B12 contains a central cobalt atom (MacPherson and Dixon, 2003). Synthetic organic minerals are produced in an attempt to increase the utilization of dietary minerals. By complexing metal ions with a variety of organic ligands, an effort is made to enhance mineral absorption across the intestinal mucosa. When thinking of the effectiveness of synthetic mineral complexes for increasing mineral availability, the concepts of stability constant and ligand molecular weight must be considered. The stability constant is a measure of the affinity between a metal ion and a ligand (Nelson, 1988). The importance of stability constant and ligand molecular weight when evaluating a synthetic metal complex. The stability constant must be high enough to allow intact absorption of the metal-ligand complex and low enough to allow metal ion removal at the metabolic point of use. In addition, the ligand molecular weight must be low enough to permit intact absorption of the metal complex (Herrick 1993).

2.4.1 Chelated trace minerals in broiler production

Chelated trace minerals have been used in broiler feeds for some time, showing promise in improving live performance, bird health, processing yield and meat quality characteristics (Dieck,, *et al.*, 2003; Bao, *et al.*, 2006). The most common trace minerals complexed include zinc, manganese, copper and iron (Koutsos, 2011). Zinc sources have studied by number of researchers who have reported improvements in broiler growth rate and feed conversion ratio with chelated zinc sources (Sanford and Kawchumnong, 1972; Sandford, 1976; Hess, *et al.*, 2001) in broiler's diets.

In addition to improvements in body weight and feed conversion, foot pad quality has been enhanced with chelated zinc (Hess *et al.*, 2001). This use of chelated zinc has gained increased importance as reported by Dawkin, *et al.*, (2004) that the measurement of foot pad lesion incidence has improved broiler welfare.

Interest is also building in using chelated trace minerals in place of a portion of the feeding inorganic mineral supplement in order to get maximum growth and health with lower levels of mineral intake, thus lowering the amount of minerals excreted from the birds (Bao *et al.*, 2006). Reducing mineral levels in litter placed on the land is an issue in many poultry industries and lower levels of complexed trace minerals may aid in reducing litter mineral excess (Paik, 2001).

Copper, Manganese or Zinc Methionine Hydroxyl Analogue (MHA) chelates (MINTREX®, Novus Intl., USA) is formed with two moles of MHA (2-hydroxyl-4-methylthiobutanoic acid as HMTBa) to one mole of trace mineral having a chelated molar ratio of one mole of metal to two moles of HMTBa to form a covalent bonds and this has been proved to have higher bioavailability than sulfate forms in broiler (Yan and Waldroup, 2006; Yi *et al.*, 2007). The HMTBa has a higher potential to be converted to methionine in the tissues of the animal (Yi *et al.*, 2007). Yi *et al*. (2007) also reported that MHA from Mintrex was fully available as a methionine source, because it can be converted to L-methionine, which is well known as the first limiting amino acid in laying hen diets (Schuttea *et al.*, 1983), within the body. In addition, MHA chelates have higher chelation strength compared with mineral complexes which indicates a higher bioavailability (Li *et al*., 2004).

2.5 Bird health and minerals effect

A number of minerals have been shown to play crucial roles in broiler health and organic trace minerals have been shown to have a role in boosting cellular and humoral immunity in broilers (Kidd *et al*., 1994; Rink and Haase, 2007). Chelated zinc compounds have shown benefits in improving immunity in birds (Pimental *et al*., 1991a; Kidd *et al*., 1994). In addition, chicks hatched from breeder hens fed chelated trace minerals have shown improved cellular and humoral immunity as well (Kidd *et al*., 1992; Kidd *et al*., 1993). Downs, *et al*., (2003) reported that the improvements in the amount of cellulitis (IP) associated with the feeding of chelated zinc products (Downs *et al*., 2000; Downs *et al*., 2003) to broiler chicken. The improvement in skin quality and healing of wounds often occurred with chelated zinc sources, or may be due to improvements in immune function (Rink and haase, 2007).

2.6 Minerals interaction

Mineral interaction is defined as the interrelationship among minerals elements reflecting through the physiological or biochemical consequences (O'Dell, 1997). The main classes of interactions are synergistic and antagonistic.

Synergism is the association of minerals that occurs largely on a metabolic level, for instance, iron and copper are synergistic in that adequate copper is needed for iron utilization (Prasad, 1978; Scheideler, 2008). Magnesium also functions in concert with potassium by enhancing its cellular retention. The synergism between calcium, magnesium and phosphorus is well known due to their requirement in the maintenance and structure of osseous tissue (Watts, 1990). Other mineral synergisms are displayed in table 1.

Table 1: Minerals synergism

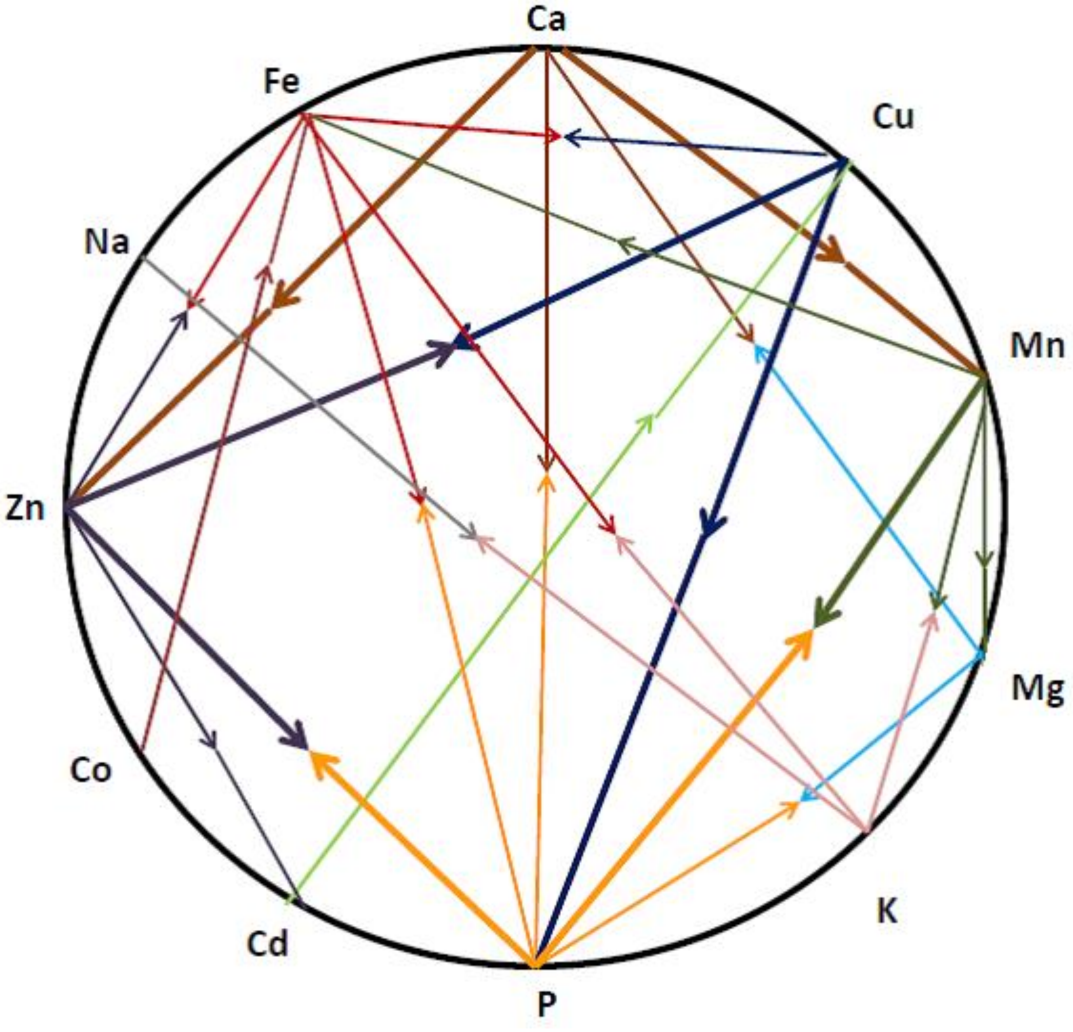
Element	Synergist Minerals
Ca	Mg-P-Cu-Na-K-Se
Mg	Ca-K-Zn-Mn-P-Cr
Na	K-Se-Co-Ca-Fe-Cu-P
K	Na-Mg-B10-Mn-Zn-P-Fe
Cu	Fe-Co-Ca-Na-Se
Zn	K-Mg-Mn-Cr-P
P	Ca-Mg-Na-K-Zn-Fe
Fe	Cu-Mn-K-Na-Cr-P-Se
Cr	Mg-Zn-K
Mn	K-Zn-Mg-Fe-P
Se	Na-K-Cu-Mn-Fe-Ca

Source: Watts, 1990

Minerals antagonism can be metabolic or absorptive but largely occur at the metabolic level. Inhibited absorption causes absorptive antagonism; that is, excess intake of a single element can decrease the intestinal absorption of another element (Richards *et al*., 2007). As an example, a high intake of calcium depresses intestinal zinc absorption, while an excess intake of zinc can depress copper absorption (Arias and Koutsos, 2005; Peterson *et al*., 2008). Antagonisms at the metabolic level occur when an excess of one element interferes with the metabolic functions of another or contributes to its excretion due to compartmental displacement (Lee *et al*., 2001; Koutsos, 2011). This occurs between zinc and copper, cadmium and zinc, iron and copper, calcium, magnesium and phosphorus (Ashmead, 1993a).

The antagonistic relationships can occur several ways within the gastrointestinal tract (Richards *et al*., 2007). The chemical reaction to form an insoluble complex, adsorbed onto the surface of colloidal particles, competition between minerals to absorption, inhibitory effects on intestinal wall processes or on the activity of some enzymes which interfere with the breakdown of feed ingredients and liberation of inorganic ions for absorption (Wedekind *et al*., 1994; Cousins *et al*., 2006; Koustsos, 2011). Mineral absorption can be affected by much interference, for instance the mineral antagonism could reduce absorption and the rate of metabolism of other minerals like calcium hinders the absorption of copper and zinc (Wedekind *et al*., 1994). Manganese also compete with iron, the intake of iron is depressed when a large amount of manganese is supplemented in diets (Sandstrom, 1992). The trace minerals bioavailability can also be improved in poultry when phytase is supplemented in diets but the action of enzymes can be restricted by dietary calcium level (Angel *et al*., 2002).

Other mineral antagonism relationships are shown in figure 1.



Source: Richards *et al*., 2007

Figure 1: Mineral Antagonists

2.7 Blood Parameters (haematology and serum biochemistry)

Haematology refers to the study of blood and its components which include blood plasma, and blood cells like Red blood cells, White blood cells and Blood platelets. Haematological parameters are Red Blood Cell count (X 106/ ul), White Blood Cell count (X104/ ul), Packed Cell Volume (%), Erythrocyte Sedimentation Rate (mm), Haemoglobin Concentration (g %), Mean Corpuscular Haemoglobin Concentration (%), and Mean Corpuscular Haemoglobin (pg) ((Islam *et al*., 2004).

The haematological status in animals can be observed through the nutritional condition, differences in food supply among the population, disease and immune suppression caused by various stresses (Cooper, 2002). However, the haematological parameters of some healthy birds are influenced by many factors which include physiological, physical and environmental conditions, water and feed restriction and nutrients conditions, environmental factors (Vecerek *et al*., 2002), fasting (Lamasova *et al*., 2004), nutritional contents (Odunsi *et al*., 1999; Kurtoglu *et al*., 2005), water and feeds restriction (Iheukwumere and Herbert, 2003), age (Furlan *et al*., 2004; Talebi *et al*., 2005), administration of drugs, breed and anti- aflatoxin premixes (Oguz *et al*., 2000).

The antibodies are serum or cell-bound proteins produced to an antigen and able to react specifically to other antigen. Some white blood cells (monocytes) have the ability to engulf the foreign particles by phagocytosis (Reeves *et al*., 2004). Copper, zinc and manganese are trace minerals which plays enormous role in many physiological and biochemical processes in animals' body. Copper as an important component of many enzymes which are critical to the maturation of hematopoietic cells and copper deficiency can result to inadequate iron utilization in organism (Reeves *et al*., 2004). Zinc is recognized as an essential mineral in erythropoiesis. Zinc plays particular catalystic role in the activity of alfa-aminolevunilic acid dehydratase which is responsible hem synthesis **(**Arcasoy, 2002). Zinc deficiency can cause adverse effect on erythropoiesis in marrow (Hughes *et al*., 2006), and a reduction of transporters and lymphocyte production (Shils *et al.,* 1997, Haddad *et al*., 2008). Manganese and iron are recognized as two main trace minerals with many similar physico-chemical properties but those minerals show antagonistic effect on each other's (Sandstrom, 1992). Manganese and iron are absorbed by binding to the same divalent metal ion transporters and therefore, a high level of manganese can result to decrease the iron absorption, thus anemia occurs (Garrick *et al*., 2003). On the other hand, high level of manganese prevents iron metabolism by pressuring the synthesis of aminolevulin at which has special role in the hem synthesis (Ivan *et al*., 1980, Maines, 1980).

The inclusion or supplementation of the trace minerals increase serum cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) (Goodwin *et al*., 1985; Konjufca *et al*., 1997) but sometimes the other parameters of serum biochemistry may have no effect when trace minerals are supplemented (Samman *et al*., 1988, Lee *et al*., 2001)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

The experiment was carried out at the broiler section (unit) of the Directorate of University Farm (DUFARM), College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Ogun State. The location lied on Latitude $7^0$13’32” N and longitude $3^0$26’04” E (Googles Earth, 2017) and 76m above sea level and located in the Tropical Rain Forest vegetation zone with an average temperature of 34.70^0C and relative humidity of 82%.

3.2 Experimental birds and management

A total of 300 unsexed day old Arbor Acre (AA) broiler chicks were obtained from CHI Ajanla Hatchery and used for the study. Brooding was done for 14 days during which the temperature of the brooding environment was maintained close to the body temperature of the broiler chicks. Temperature was maintained at 36 °C for the first 0 to 2 days and then reduced gradually by 2 °C in every five days to ambient temperature at the last week of brooding. Feed and clean water were supplied *ad libitum*. The birds were brooded and reared intensively in two phases diets (between 0-3 and 4-6 weeks) in a battery cage system for a period of six (6) weeks. A completely randomized design was used in allocating birds in the treatments. The broilers were allocated to five (5) treatments and a treatment was divided into six (6) replicates and each replicate with ten (10) birds making total of sixty (60) birds in a treatment. The health management of the birds was closely monitored with the adequate supply of the prophylactics and vaccines at time due. The preventive measure like proper biosecurity and regular sanitization was also observed throughout the experimental period. The crude protein (CP) and metabolizable energy (ME) contents of the feeds for each growth phase were balanced within the recommended range (NRC, 1994) Table 3. Feed and water were given *ad libitum* throughout the experimental period.

3.3 Test ingredients

The test ingredients used for the study:

Chelated trace minerals of Cu, Zn and Mn (Mintrex®) containing 15% Cu from Cu(2-hydroxyl-4-methylthiobutanoic acid as $HMTBa)_2$, 16% Zn from $Zn(HMTBa)_2$ and 13% Mn from $Mn(HMTBa)_2$ were obtained from Novus Inc., USA.

The inorganic sources of Cu, Zn and Mn were purchased from an inorganic manufacturer in Lagos. The composition of the inorganic sources used are described as follow; copper sulphate pentahydrate ($CuSO_4.7H_2O$: containing 25% Cu), zinc oxide (ZnO: containing 72% Zn) and manganese dioxide (MnO_2: containing 64% Mn) were used as inorganic supplemental Cu, Zn and Mn respectively

The formulated premix used for the study was acquired from a local premix manufacturing company (Rotinol) at Gbonogun, Abeokuta Ogun state.

3.4 Experimental diets and design

The balanced diets of a complete nutritive value with respect to the NRC 1994 requirement were supplied but with different levels of supplemental trace minerals inclusion to all treatments from day old to 6 weeks of age. The experimental birds (Arbor Acre broiler) were fed *ad-libitum* with different supplemental diets of trace mineral sources in each treatment. The five (5) treatments diets with varying supplemental level of inorganic trace minerals (ITM) and chelated trace minerals (CTM) is shown in Table 2 below. The treatment one (1) diet was a negative control while other treatment 2 (AA manufacturer recommendation level of TM), 3, 4 and 5 were diets with lower supplemental level of ITM and CTM respectively.

Table 2: Treatments with supplemental level of ITM and CTM

Treatment	Composition
Control (NC)	Basal Diet (**BD**)
100% ITM	15, 100 and 100 mg/kg of inorganic Cu, Zn and Mn respectively (**100% ITM supplemental level**)
50% ITM	7.5, 50 and 50 mg/kg of inorganic Cu, Zn and Mn respectively (**50% ITM supplemental level)**
50% CTM	7.5, 50 and 50 mg/kg of chelated Cu, Zn and Mn respectively (**50% CTM supplemental level)**
25% CTM	3.75, 25 and 25 mg/kg of chelated Cu, Zn and Mn respectively (**25% CTM supplemental level)**

NC= negative control
100% ITM (PC) =positive control (AA manufacturer recommendation level of trace mineral)
ITM= inorganic trace mineral
CTM= chelated trace mineral
BD= basal diet
TM= trace mineral

Table 3: Gross Composition of Experimental Basal diets

INGREDIENTS (kg)	(0 – 3 weeks)	(4 – 6 weeks)
Maize	54	65
Soya meal	40	30.7
Wheat Bran	2	1.0
Lime stone	1.5	1.0
Bone meal	1.2	1.0
Fish meal (72% CP)	0.5	0.5
Methionine Hydroxyl Analog (MHA)[a]	0.25	0.2
Lysine	0.2	0.2
Salt	0.15	0.15
Vitamin-Mineral Premix (Cu, Zn & Mn free)[b]	0.25	0.00
Vitamin-Mineral Premix (Cu, Zn & Mn free)[c]	0.00	0.25
TOTAL	**100**	**100**
DETERMINED ANALYSIS FOR EXPERIMENTAL DIETS		
Dry matter (%)	88.9	94
Crude protein (%)	23.70	19.20
ME (KCal/kg)	2946.23	3054.90
Nitrogen free extract (%)	47.02	55.6
Crude Fibres (%)	4.11	4.48
Ether extract (%)	5.23	4.92
Ash content (%)	8.84	9.8

[a] Methionine Hydroxyl Analog (MHA)**:** (Novus International Inc., St. Charles, MO), feed supplement providing 84% Methionine activity.

[b] Vitamin - mineral premix: (Rotinol) based on 2.5 kg / ton.(Thiamine, 2000 mg; riboflavin, 7000 mg; pyridoxine, 5000 mg; cyanocobalamine, 1700 mg; niacin, 30 000 mg; D-panthotenate 10,000; folic acid, 800 mg; biotin, 2000 mg; Retinyl acetate, 12,000, 000 i.u; cholecalciferol, 2,400, 000 i.u; tocopherol acetate, 35 000 i.u; menadione, 4000 mg; ascorbic acid 60,000mg; manganese, nil; iron, 70,200 mg; zinc, nil; copper, nil; cobalt, 200 mg; iodine, 400 mg; selenium, 80 mg; choline chloride, 500 000 mg)

ME= metabolizable energy

[c] Vitamin - mineral Premix: (Rotinol) based on 2.5 kg / ton. (Thiamine, 1000 mg; riboflavin,

6000 mg; pyridoxine, 5000 mg; cyanocobalamine, 25 mg; niacin, 60 000 mg; D-panthotenate, 20 000 mg; folic acid, 200 mg; D-biotin, 8 mg; Retinyl acetate, 40 000 mg; cholecalciferol, 500 mg; tocopherol acetate, 40 000 mg; menadione, 800 mg; manganese, nil; iron, 80 000 mg; zinc, nil; copper, nil; cobalt, 80 mg; iodine, 400 m; selenium, 40 mg; choline chloride,80000mg)
CP = crude protein

Table 4: **ANALYSED TRACE MINERALS COMPOSITION FOR EXPERIMENTAL DIETS (mg/kg)**

	DIETS (0 – 3 weeks)				
	Control	**ITM**		**CTM**	
Mineral sources	**(BD)**	**100%**	**50%**	**50%**	**25%**
Cu (inorganic)	7.2	20.1	12.3	0	0
Zn (inorganic)	19.8	107.2	61.2	0	0
Mn (inorgnic)	9.1	104.5	81.4	0	0
Cu (chelated)	0	0	0	14.0	7.2
Zn (chelated)	0	0	0	62.0	38.8
Mn (chelated)	0	0	0	58.2	32.2
	DIETS (4 - 6 weeks)				
Cu (inorganic)	7.6	17.1	10.9	0	0
Zn (inorganic)	24.6	109.1	66.7	0	0
Mn (inorganic)	7.9	105.7	82.2	0	0
Cu (chelated)	0	0	0	9.6	8.8
Zn (chelated)	0	0	0	65.7	40.6
Mn (chelated)	0	0	0	61.1	30.3

3.5 GROWTH PERFORMANCE DATA

The average body weight and the feed intake of AA broiler in each replicate were recorded on a weekly basis. The mortality from each replicate on a daily basis was recorded and adjusted when calculating other parameters. The relationship between average body weight, weight gain, feed intake and feed conversion ratio were closely observed and recorded on a weekly basis.

$$\textbf{Average Body Weight (g)} = \frac{\text{Total weight of the birds}}{\text{Total Number of birds weighed}}$$

Weight Gain (g) = final body weight – initial body weight

Feed intake (g) = Weight of feed given – weight of left over feed

$$\textbf{Feed Conversion Ratio (FCR)} = \frac{\text{Total weight of the feeds consumed}}{\text{Total weight gain}}$$

3.6 NUTRIENT DIGESTIBILITY

The digestibility trial was conducted between the 32nd and 35th days of the experiment. Thirty (30) birds (each bird per replicate) were used for the digestibility study. The birds were transferred to a metabolic cage and allowed to acclimatize to the environment for a period of 72 hours before collection of excreta. Birds in each treatment were fed with their respective diets. The records of feed intake and fresh weight of excreta from the respective groups were taken on a daily basis for a period of 4 days using the total collection method. The collected droppings were weighed and dried at 70^0C to a constant weight. The dried excreta samples were taken to the laboratory for proximate composition analysis as outlined by AOAC (2005).

Apparent nutrient digestibility will be computed as follows:

$$\text{Nutrient digestibility} = \frac{\text{Nutrient intake} - \text{Nutrient voided}}{\text{Nutrient intake}} \times 100$$

Nutrient intake = Nutrient in feed X feed intake

Nutrient voided = Nutrient in excreta X Quantity of excreta voided

3.7 BLOOD COLLECTION

At day 42nd, blood samples were collected from 30 birds (a bird from each replicate) at the experimental broiler unit through the wing vein. 2mL of blood were drawn from the wing vein using sterile syringe and needle and deposited in the sample bottles (ethylenediamine-tetra-acetate as EDTA and plain bottles). The blood samples collected were taken to laboratory for blood analysis and these were carried out at the Apex Diagnostic Laboratory, Alogi, Abeokuta, Ogun State.

EDTA was used to prevent blood clotting for haematology analysis. 2 mL of blood were collected and deposited into tubes containing ethylenediamine-tetra-acetate (EDTA) and another 2 mL were collected into plain bottles. The blood in the EDTA bottles was used to determine haematological parameters including packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC), haemoglobin (Hb), neutrophil, lymphocyte, monocyte, eosinophil and basophil while that in the plain bottles were used to determine serum parameters (serum total protein, albumin, globulin, uric acid, creatinine, serum (Cu, Zn, Mn), serum lipids protein (cholesterol) and enzymes. The PCV was determined by microhaematocrit method (Baker and Silverton, 1985) and Hb and RBC were determined using colorimetry cyanomethaemoglobin and improved Neubauer haemocytometer methods respectively (Jain, 1986). The serum total protein, albumin and globulin were analysed colorimetricaly using a diagnostic reagent kit (Varley et al., 1980). Plasma was separated from the blood in the EDTA bottles with a micropipette into a test tube for triglyceride and cholesterol analysis. The cholesterol and triglyceride assay of the blood plasma were done using Randox methods.

3.8 CHEMICAL ANALYSES AND LABORATORY ACTIVITIES

3.8.1 Proximate analysis

A.O.A.C (2005) method was used to carry out the chemical analysis (proximate) on the samples of experimental diets and excreta.

3.8.2 Determination of mineral concentrations in tissues and sera

Wet ash method using a nitric per chloric acid mixture was used to digest liver and kidney (Yokoi *et al*., 1990). Serum was digested in concentrated nitric acid (Mohanna and Nys, 1999). Cu, Zn and Mn in control group, Cu in Cu-MHA group, Zn in Zn-MHA group and Mn in Mn-MHA group were analyzed by a flame atomic-absorption spectrometer.

3.9 STATISTICAL ANALYSIS

Data collected were subjected to one – way Analysis of Variance (ANOVA) technique in a Completely Randomized Designs using General Linear Model procedure of SAS (2007). The means of significant results were compared by Tukey’s HSD Test in the same package. The body weight after the third (3rd) week data collected were subjected to Co-Variate Analysis.

Experimental Model

The model for this experiment is outlined below:

$$Yijk = \mu + Ti + \sum ij$$

Where

$Yijk$ = yield/output

μ – population mean

Ti – Effect of ith source of trace minerals

$\sum ij$ – random residual error

CHAPTER FOUR

4.0 RESULTS

4.1 Growth Performance of the broiler chickens fed supplemental diets of different level of ITM and CTM (21 days)

The effect of supplemental inorganic and chelated trace minerals on the growth performance of broiler chickens is shown in Table 5. The groups fed 100% (15, 100 and 100mg/kg of Cu sulfates, Zn and Mn oxides respectively) supplemental diets of ITM had higher ($p<0.05$) final body weight of 607.58 g, daily feed intake of 41.91 g and daily weight gain of 27.92 g than the other supplemental diets groups while the group fed basal diet had the lowest ($p<0.05$). However, there was no difference ($p>0.05$) in final body weight, daily weight gain, FCR and feed cost per bird between the groups fed 100% ITM level and 50% CTM level. Although there was a slightly lower ($p<0.05$) final body weight of 552.13g from the group fed 25% CTM supplemental level (3.75, 25 and 25mg/kg of Cu, Zn and Mn chelated with HMTBa respectively) compared with the group fed 100% ITM level (607.58g). The groups fed 50% and 25% supplemental diet of CTM had higher ($p<0.05$) daily weight gain (25.88g and 24.39g respectively) than the group fed basal diet (BD) (22.49g). The group fed 50% supplemental level of CTM had a lower ($p<0.05$) daily feed intake of 39.79g than the group fed 100% ITM level (41.91g) while the groups fed basal diet and the supplemental diet of 25% CTM level had lowest ($p<0.05$) daily feed intake of 38.2g and 38.92g respectively. The group fed basal diet had a lowest ($p<0.05$) FCR of 1.68 than the other groups while the groups fed 100% ITM and 50% CTM supplemental diets level have highest ($p<0.05$) FCR of 1.55 and 1.54 respectively. The feed cost per bird for group fed BD (₦99.63) was significantly lower ($p<0.05$) compared with the groups fed 50% and 25% supplemental diet of CTM (₦113.63 and ₦106.34 respectively) but the group fed 100% supplemental level of ITM had the lowest ($p<0.05$) feed cost per weight gain than other groups.

Table 5: Effect of inorganic and chelated trace minerals on growth performance (21 days)

PARAMETERS	Control (BD)	ITM		CTM		SEM	*P* –Value
		100%	50%	50%	25%		
IBW (g/bird)	40.18	40.20	40.22	40.24	40.01	0.04	0.31
FBW (g/bird)	512.41^{c}	607.58^{a}	568.67^{b}	583.64^{ab}	552.13^{b}	6.81	0.00
Daily Feed Intake (g/bird/day)	38.20^{c}	41.91^{a}	40.17^{b}	39.79^{b}	38.92^{bc}	0.28	0.00
Daily Weight Gain (g/bird/day)	22.49^{c}	27.02^{a}	25.16^{b}	25.88^{ab}	24.39^{b}	0.32	0.00
FCR	1.68^{a}	1.55^{b}	1.59^{ab}	1.54^{b}	1.60^{ab}	0.01	0.01
Feed Cost/bird (₦)	99.63^{d}	110.38^{ab}	105.27^{c}	113.63^{a}	106.34^{bc}	0.98	0.00
Cost/Weight Gain (₦/g)	0.21^{a}	0.19^{b}	0.20^{ab}	0.21^{a}	0.21^{a}	0.00	0.01
Livability (%)	96.67	100	98.33	98.33	100	0.63	0.21
Lameness (%)	3.33	1.67	0.00	0.00	0.00	0.56	0.11

^{a,b,c,d} Means in the same row having different superscripts are significantly different at $p < 0.05$.
SEM = Standard error of mean
IBW= Initial body weight
FBW= final body weight
FCR= feed conversion ratio
Control = Basal diets (BD)
100% ITM = 100% ITM supplemental diets
50% ITM = 50% ITM supplemental diets
50% CTM = 50% CTM supplemental diets
25% CTM = 25% CTM supplemental diets

4.2 Growth Performance of the broiler chickens fed supplemental diets of different level of ITM and CTM (21 days)

In Table 6, the groups fed 100% and 50% supplemental diets of ITM and CTM level respectively had highest ($p<0.05$) final body weight and daily weight gain of 2151.83 g, 73.54 g and 2127.67 g, 73.53 g respectively compared to the other groups fed basal diet, 50% ITM and 25% CTM supplemental level diet. The group fed 50% CTM supplemental level had better ($p<0.05$) FCR and higher feed cost per bird of 1.91 and ₦389.69 g respectively. The daily feed intake (146.29 g) for the group fed 100% ITM supplemental level was higher ($p<0.05$) than the other groups. Also, there was a significant lower ($p<0.05$) values in all growth performance parameters for the group fed BD compared with the other trace minerals supplemental groups. The groups fed 100% ITM and 50% CTM level had higher ($p<0.05$) final body weight of 2151.83g and 2127.67g respectively compared with groups fed 50% ITM and 25% CTM level (1963.33g and 1993g) but at the other hand there was no significant effect ($p>0.05$) between the groups fed 100% and 50% supplemental diets of ITM and CTM respectively on all the growth performance parameters except the feed cost per bird that was higher ($p<0.05$) for group fed 50% CTM supplemental level.

Table 6: Effect of inorganic and chelated trace minerals on growth performance (42 days)

PARAMETERS	**Control**	**ITM**		**CTM**		**SEM**	***P* –Value**
	(BD)	**100%**	**50%**	**50%**	**25%**		
FBW (g/bird)	1875.17^{b}	2151.83^{a}	1963.33^{b}	2127.67^{a}	1993.00^{b}	23.47	0.00
Daily Feed Intake (g/bird/day)	134.80^{b}	146.29^{a}	132.96^{b}	140.18^{ab}	136.27^{b}	1.33	0.01
Daily Weight Gain (g/bird/day)	64.89^{b}	73.54^{a}	66.41^{b}	73.53^{a}	68.61^{ab}	0.88	0.00
FCR	2.08^{a}	1.99^{ab}	2.00^{ab}	1.91^{b}	1.99^{ab}	0.02	0.02
Feed Cost/bird (₦)	341.40^{c}	374.18^{ab}	338.40^{c}	389.69^{a}	361.99^{bc}	4.44	0.00
Cost/Weight Gain (₦/g)	0.25	0.24	0.24	0.25	0.25	0.00	0.18
Livability (%)	91.30	93.33	93.15	96.67	96.56	1.18	0.22
Lameness (%)	7.04	5.19	6.67	0.00	1.67	1.18	0.13

^{a,b,c,d} Means in the same row having different superscripts are significantly different at $p < 0.05$.
SEM = Standard error of mean
FBW= final body weight
FCR= feed conversion ratio
Control = Basal diets (BD)
100% ITM = 100% ITM supplemental diets
50% ITM = 50% ITM supplemental diets
50% CTM = 50% CTM supplemental diets
25% CTM = 25% CTM supplemental diets

4.3 Effect of inorganic and chelated trace minerals blend on trace mineral digestibility coefficient of the broiler chickens

In Table 7, the trace mineral (Cu) excreted was slightly lower ($p<0.05$) for the group fed BD (0.17mg/100g) as well as lower ($p<0.05$) excreta Zn and Mn of 0.67 mg/100g and 0.07 mg/100g respectively than the other groups fed supplemented ITM and CTM. The excreta Zn (1.03 mg/100g) for group fed 100% ITM level was significantly higher ($p<0.05$) than 0.86mg/100g and 0.93mg/100g for the groups fed 50% and 25% supplemental diet of CTM respectively. The group fed 50% supplemental diet of CTM had a significant lower ($p<0.05$) excreta Zn of 0.86 mg/100g than the group fed 50% ITM level of 0.98 mg/100g excreta Zn. Similarly, the highest ($p<0.05$) Mn of 0.29mg/100g excreted for the group fed 100% supplemental ITM level than the other groups fed 50%, 50% and 25% supplemental diets of ITM, CTM and CTM respectively. However, there was no significant difference ($p>0.05$) of Mn excreted between the groups fed 50% supplemental level of ITM and 50% supplemental level of CTM.

Table 7: Effect of inorganic and chelated trace minerals blend on trace mineral digestibility coefficient of the broiler chickens (42 days)

Parameters	**Control**	**ITM**		**CTM**		**SEM**	***P*-Value**
	(BD)	**100%**	**50%**	**50%**	**25%**		
Excreta (mg/100g)							
Cu	0.17^{b}	0.34^{a}	0.34^{a}	0.30^{a}	0.32^{a}	0.29	0.00
Zn	0.67^{d}	1.03^{a}	0.98^{ab}	0.86^{c}	0.93^{b}	0.89	0.00
Mn	0.07^{c}	0.29^{a}	0.23^{b}	0.19^{b}	0.20^{b}	0.19	0.00
DM (%)	18.26	18.81	17.28	20.56	20.56	0.57	0.09

^{a,b,c,} Means in the same row having different superscripts are significantly different at ($p < 0.05$.)
SEM = Standard error of mean
DM = dry matter
Control = Basal diets (BD)
100% ITM = 100% ITM supplemental diets
50% ITM = 50% ITM supplemental diets
50% CTM = 50% CTM supplemental diets
25% CTM = 25% CTM supplemental diets

4.4 Effect of inorganic and chelated trace minerals on haematological parameters of the experimental broiler chickens (42 days)

In Table 8, the groups fed 50% and 25% supplemental level of CTM had higher ($p<0.05$) packed cell volumes (34.67% and 33.67%) and haemoglobin of 11.33 g/dL and 11.20 g/dL respectively than the other supplemental ITM groups and the group fed BD (28.5% and 9.57g/dL respectively). The white blood cell of the group fed 50% CTM level (11.07x10$^{9/L}$) was significantly lower ($p<0.05$) compared to the other groups and it also had a significant higher ($p<0.05$) red blood cell (13x10$^{9/L}$) while the groups fed 100% ITM, 50% ITM level and BD had 2.80x10$^{9/L}$, 2.89x10$^{9/L}$ and 2.42x10$^{9/L}$ (the lowest) RBC respectively. The group fed BD had the highest ($p<0.05$) neutrophil (51%) and the lowest ($p<0.05$) lymphocyte (46%) compared to the other groups fed supplemental diet of ITM and CTM. No significant difference ($p>0.05$) was observed on monocytes, basophil and eosinophil among the groups.

Table 8: Effect of inorganic and chelated trace minerals on haematological indices of the experimental broiler chickens (42 days)

PARAMETERS	Control (BD)	ITM		CTM		SEM	*P* –Value
		100%	50%	50%	25%		
PCV (%)	28.50^{b}	31.00^{ab}	32.33^{ab}	34.67^{a}	33.67^{a}	0.66	0.00
Hb (g/dL)	9.57^{b}	10.33^{ab}	10.67^{ab}	11.33^{a}	11.20^{a}	0.21	0.02
WBC (10^6/mm^3)	17.97^{a}	17.43^{a}	18.67^{a}	11.07^{b}	13.90^{ab}	0.92	0.01
RBC (10^6/mm^3)	2.42^{c}	2.80^{b}	2.89^{ab}	3.12^{a}	2.98^{ab}	0.07	0.00
Neutrophil (%)	51.00^{a}	26.00^{b}	27.33^{b}	15.67^{b}	14.00^{b}	3.73	0.00
Lymphocyte(%)	46.00^{b}	70.67^{a}	69.00^{a}	80.00^{a}	81.33^{a}	3.71	0.00
Monocyte (%)	2.00	1.33	2.67	3.00	2.67	0.41	0.77
Eosinophil (%)	1.00	1.67	1.00	1.00	1.33	0.34	0.98
Basophil (%)	0.00	0.33	0.00	0.33	0.67	0.15	0.68

^{a,b,c} Means in the same row having different superscripts are significantly different at ($p < 0.05$.)
SEM = Standard error of mean;
WBC= white blood cell;
RBC= red blood cell;
PCV= packed cell volume
Hb= haemoglobin
Control = Basal diets (BD)
100% ITM = 100% ITM supplemental diets
50% ITM = 50% ITM supplemental diets
50% CTM = 50% CTM supplemental diets
25% CTM = 25% CTM supplemental diets

4.5 Effect of inorganic and chelated trace minerals of serum biochemistry of the experimental broiler chickens

In Table 9 below, the group fed 50% supplemental diet of CTM had the largest ($p<0.05$) total protein (73.2g/L), albumin (41.20 g/L) and globulin (32 g/L) in serum than the other groups but the group fed 100% ITM level had the lowest ($p<0.05$) serum protein of 62.53g/L. Also, the group fed BD had a significantly lower ($p<0.05$) albumin (35.77g/L) compared with 41.2g/L of the group fed 50% supplemental diet of CTM. The serum Cu (16.5ug/dL), Zn (14.13ug/dL) and Mn (1.88ug/dL) were significantly higher ($p<0.05$) for the group fed 25% supplemental diet of CTM compared with the group fed 100% supplemental ITM level with 15.08, 11.03, and 1.61ug/dL of serum Cu, Zn and Mn respectively while the group fed BD had the lowest ($p<0.05$) Cu, Zn and Mn in the serum.

From Table 10, the group fed 25% supplemental diet of CTM had a slightly higher ($p<0.05$) high density lipid (HDL) of 50.87mg/dL than the other groups fed supplemental diet of 100% ITM, 50% ITM, 50% CTM level and the control group with HDL of 27.57, 35.77, 38.70, and 32.10 mg/dL respectively. However, there was no significant difference ($p>0.05$) among the groups on the other parameters in Table 10 below.

Table 9: Effect of inorganic and chelated trace minerals of serum biochemistry of the experimental broiler chickens (42 days)

PARAMETERS	Control (BD)	ITM		CTM		SEM	*P* –Value
		100%	50%	50%	25%		
TP (g/L)	65.90^{c}	62.53^{d}	67.00^{bc}	73.20^{a}	69.87^{b}	0.99	0.00
Albumin (g/L)	35.77^{c}	36.67^{bc}	38.30^{bc}	41.20^{a}	39.73^{ab}	0.60	0.00
Globulin (g/L)	30.27^{ab}	25.87^{c}	28.70^{b}	32.00^{a}	30.13^{ab}	0.58	0.00
Creatinine (mg/dL)	0.77	1.40	1.27	0.87	1.43	0.11	0.17
Uric (mg/dL)	5.80	8.57	8.33	7.93	7.60	0.41	0.21
Serum Cu (ug/dL)	6.77^{c}	15.08^{b}	15.53^{b}	16.17^{a}	16.50^{a}	0.97	0.00
Serum Zn (ug/dL)	4.05^{e}	11.03^{d}	12.80^{c}	13.53^{b}	14.13^{a}	0.98	0.00
Serum Mn (ug/dL)	0.62^{d}	1.61^{c}	1.78^{b}	1.84^{ab}	1.88^{a}	0.13	0.00

^{a,b,c,d,e} Means in the same row having different superscripts are significantly different at $p < 0.05$.
SEM = Standard error of mean
TP= total protein
Control = Basal diets (BD)
100% ITM = 100% ITM supplemental diets
50% ITM = 50% ITM supplemental diets
50% CTM = 50% CTM supplemental diets
25% CTM = 25% CTM supplemental diets

Table 10: Effect of inorganic and chelated trace minerals on serum lipid, protein and enzymes of the experimental broiler chickens

PARAMETERS	Control	ITM		CTM		SEM	*P* –Value
	(BD)	100%	50%	50%	25%		
Chol (mg/dL)	126.77	157.03	124.20	127.73	127.37	5.33	0.27
Trig (mg/dL)	77.57	95.87	78.23	84.50	80.77	4.01	0.66
HDL (mg/dL)	32.10^{b}	27.57^{b}	35.77^{b}	38.70^{ab}	50.87^{a}	2.35	0.00
LDL (mg/dL)	79.10	110.30	69.83	75.07	60.33	6.22	0.09
VLDL (mg/dL)	42.30	59.43	45.73	51.13	41.00	3.06	0.33
AST (IU/L)	223.80	215.03	264.20	228.67	228.43	7.91	0.37
ALT (IU/L)	16.07	21.97	24.80	31.13	10.33	2.76	0.13

^{a,b}Means in the same row having different superscripts are significantly different at ($p < 0.05$.)
SEM = Standard error of mean
HDL=high density lipid
LDL= low density lipid
VLDL= very low density lipid
AST= aspartate transaminase
ALT=alanine transaminase
Control = Basal diets (BD)
Chol = cholesterol
Trig = triglyceride
100% ITM = 100% ITM supplemental diets
50% ITM = 50% ITM supplemental diets
50% CTM = 50% CTM supplemental diets
25% CTM = 25% CTM supplemental diets

4.6 Effect of ITM and CTM on tissue (liver and kidney) minerals concentrations

In Table 11, the group fed BD had a lower ($p<0.05$) Cu level of 0.38mg/100g in liver than the other groups fed supplemental diet of ITM or CTM. The liver Zn (4.22mg/100g) and Mn (0.50mg/100g) of the group fed 25% supplemental diet of CTM were higher ($p<0.05$) than the group fed 100% supplemental diet of ITM (3.64 and 0.42mg/100g). Also, the Cu level of 1.17mg/100g in kidney was significantly higher ($p<0.05$) at the group fed 25% supplemental diet of CTM, followed by the group fed 50% CTM level in diet compared with the group fed 100% supplemental diet of ITM with a lower ($p<0.05$) Cu level (0.76mg/100g) in Kidney. Furthermore, the Zn level of 7.12mg/100g in kidney of the group fed 100% ITM level was slightly lower ($p<0.05$) than the groups fed supplemental diet of ITM or CTM. However, in kidney Mn level, there was no significant difference ($p>0.05$) among the groups fed supplemental diet of ITM or CTM except the group fed BD with the lowest ($p<0.05$) Mn level of 0.06mg/100g in kidney.

Table 11: Trace mineral concentration of tissue (liver and kidney) of the broiler (at day 42) fed diet of varying supplemental level of inorganic and chelated trace minerals

PARAMETERS	Control	ITM		CTM		SEM	*P* –Value
	(BD)	100%	50%	50%	25%		
Liver (mg/100g)							
Cu	0.38^{b}	1.14^{a}	1.41^{a}	1.56^{a}	1.63^{a}	0.14	0.00
Zn	0.96^{d}	3.64^{c}	3.87^{b}	3.99^{b}	4.22^{a}	0.32	0.00
Mn	0.09^{e}	0.42^{c}	0.39^{d}	0.47^{b}	0.50^{a}	0.04	0.00
Kidney (mg/100g)							
Cu	0.32^{e}	0.76^{d}	0.96^{c}	1.09^{b}	1.17^{a}	0.08	0.00
Zn	1.46^{c}	7.12^{b}	7.77^{a}	7.97^{a}	7.88^{a}	0.67	0.00
Mn	0.06^{b}	0.22^{a}	0.19^{ab}	0.24^{a}	0.26^{a}	0.02	0.01

^{a,b,c} Means in the same row having different superscripts are significantly different at ($p < 0.05$.)
SEM = Standard error of mean
Control = Basal diets (BD)
100% ITM = 100% ITM supplemental diets
50% ITM = 50% ITM supplemental diets
50% CTM = 50% CTM supplemental diets
25% CTM = 25% CTM supplemental diets

CHAPTER FIVE

5.0 DISCUSSION

The reduction in feed intake as trace mineral supplementation reduced could be a nutritional response to trace mineral supplementation. The higher zinc supplementation in diets influenced the increase in feed intake of broiler chickens and this could also be attributed to its role in activating glycolysis. It has been reported that zinc deficiency suppressed feed intake in broilers fed various levels of trace minerals (Bao *et al*., 2010). The finding in this study supports this report. The improvement in the weight gain and FCR observed in this study was due to trace mineral supplementation which could have been as a result of improved feed intake and the ability of birds fed diet supplemented with trace mineral to utilize nutrient efficiently. This is in agreement with the work of Nollet *et al*., (2008) who reported an improved weight gain and FCR in broilers fed diet supplemented with trace minerals between 11 – 21 days of age. This study however disagrees with report of Nollet *et al*., (2007) who reported that the supplemental trace minerals of Cu, Zn and Mn (ITM or CTM) had no effect on the growth performance of broiler chickens. This could be as a result of environmental variation (tropics) and breed of the birds. Higher supplementation level of zinc in group fed 100% ITM level as compared to the BD group between 4 – 6 weeks could have resulted to the increased feed intake. This finding is in agreement with Bao *et* al., (2010) who reported a depressed feed intake as a result of zinc deficiency. Better growth performance recorded in groups fed 100% ITM and 50% CTM supplemental level between week 4 and 6 could be adduced to the higher supplementation level of copper in the diet which might have improved weight gain. The higher copper availability plays vital roles in improving growth performance, as copper contains antibacterial properties or antibiotic growth promoters (Leeson, 2009). This study agrees with the report of Torki *et al*., (2014) who observed a better weight gain in birds fed diet supplemented with copper. The increased in feed cost per bird in groups fed 100% ITM and 50% CTM supplemental level could be traced to the cost of supplemental trace minerals coupled with the quantity of feeds consumed

The decrease in trace minerals (Zn, Cu, Mn) from the analysed faecal samples of the groups fed diets supplemented with CTM is attributed to the protection of these trace minerals by the chelated 2- hydroxyl -4- methyl thiobutanoic acid (HMTBa) at the GIT and intestinal wall which made these CTMs to be more bioavailable for absorption. This study is in line with that of Nollet *et al*., (2007) who reported that chelated minerals were better absorbed in the monogastric enterocyte and the higher availability of chelates may be connected to the shielding of the minerals positive charge during chelation. It was also stated that the minerals that were chelated to small peptides have much greater bioavailability through increased selective transport of peptides at gut level (Webb *et al*., 2005). Manangi *et al*., (2012) also reported that at day 54, Zn, Cu, and Mn from deep litter of birds fed Zn-, Cu-, and Mn-$(HMTBa)_2$ were reduced than the litter from birds fed ITM as a result of the increased trace mineral bioavailability from the chelated source of minerals.

Higher haemoglobin and PCV concentration in blood of broiler birds fed diets supplemented with chelated trace minerals than those receiving BD reflects the importance of Cu in haemoglobin synthesis. This indicates that Cu was more bioavailable in the CTM groups since Cu and Fe are known to play vital roles in the synthesis of haemoglobin and for the synthesis of enzyme needed for normal metabolism (Close, 1999). The increased WBC of birds given BD relative to those receiving CTM suggests a stimulation of the immune system by the former. This showed that the birds fed CTM supplemental diets with the lower WBC had immune stability and the birds were not challenged. This is in line with the report of Jegede *et al*., (2011) who reported higher haemoglobin and PCV with lower WBC in broilers fed Cu-proteinates. The increase in RBC triggered by trace minerals supplementation would mean that there would be more circulation of oxygen in the blood of birds fed diets supplemented with chelated trace minerals. It could lead to higher haemoglobin content in groups supplemented with trace mineral since RBC is known to be the major carrier of haemoglobin in the blood (Etim *et al*., 2014).

The improvement in total protein, serum albumin and globulin in groups fed diet supplemented with CTM as a result of the supplemented methionine hydroxyl analog (MHA) chelated with trace minerals compared to the groups fed diet supplemented with ITM. The higher serum protein, albumin and globulin show that the MHA or 2- hydroxyl -4- methyl thiobutanoic acid (HMTBa) which is regarded as precursor of DL- methionine (first limiting amino acid) (Richards *et al*., 2007) were released from the ligand. Copper is also involved in blood proteins and has capacity to influence serum protein (Chandra, 1990; Uany., *et al*, 1998) This study is supported by Corzo *et al*., (2009) who reported that supplemental amino acid (valine and isoleucine) in broiler chicken diet increased the serum protein, albumin and globulin.

The increased serum Cu, Zn and Mn of the groups fed supplemental diet of CTM compared with the groups fed ITM level and BD with lower serum minerals indicates that the CTM were more bioavailable to the broiler chickens and this also shows that there is less antagonism with other dietary minerals and among themselves. This study agrees with the report of Chowdhury *et al*. (2004) and Salami *et al.,* (2016) who reported higher bioavailability of trace mineral from mineral chelates and less antagonistic interaction of chelates with other dietary constituents in the digestive tract compared with inorganic salts.

The higher concentration of trace minerals (Cu, Zn, Mn) observed in liver and kidney of birds fed diet supplemented with 50% and 25% CTM level was due to higher bioavailability of CTM in the tissues. The bioavailability of these trace minerals was higher in the tissues of birds fed CTMs as compared to other counterpart group fed ITM level due to increased concentration in the tissues (kidney and liver). This study agrees with Salami *et al*., (2016) who reported that the accumulation of Cu and Mn in the liver and serum of turkeys following dietary supplementation with high level of CTMB. Liver Cu and Zn concentrations in turkeys fed diets with different levels of CTMB were higher compared with the turkeys fed ITMB supplemented diets. The increased bioavailability of these micronutrients in the liver when presented as mineral chelates may be due to improved absorption because of their binding to organic moieties, or to the protective effect due to their connection with chelated matrices (Close 1999). The interaction of Zn and Mn is linked with their retention in hepatic cells (Sunder *et al*., 2012).

5.1 Conclusion

1. Replacement of inorganic trace minerals with reduced levels (up to 75% reduction) of chelated trace minerals had no detrimental effect on the growth performance of broiler chickens.
2. The reduced level of supplemental chelated trace minerals (Cu, Zn, and Mn) in broiler diet improved trace mineral digestibility (absorption) and significantly reduced excreta trace minerals concentration.
3. Supplementation of CTM (Cu, Zn and Mn) up to 75% reduction of ITM improved white blood cell, haemoglobin, packed cell volume and bioavailability of serum Cu, Zn and Mn of broiler chickens.
4. The CTM (Cu, Zn and Mn) supplemented in broiler diet at a reduced inclusion level were more bioavailable in liver and kidney Cu, Zn and Mn compared with the respective inorganic trace mineral sources.

5.2 Recommendation

1. To achieve an optimum performance of the broiler chicken, the chelated trace minerals should be supplemented in diets of broiler chickens at 50% lower than the inorganic trace minerals level.
2. For cost effectiveness, CTM should be supplemented to broilers’ diet at 3.75, 25, 25 mg/kg of Cu, Zn and Mn respectively.
3. Supplemental level of CTM at 50% should be added to feed in order to reduce the trace minerals concentration in excreta which have potentials to reduce environmental pollution.

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