GROWTH, HISTOPATHOLOGY OF ORGANS AND SEMEN CHARACTERISTICS OF COCKS FED DIETARY ORGANIC ZINC SOURCE

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ABSTRACT

Most divalent minerals in plants are bound as phytate resulting in poor bio-availability in monogastrics, while inorganic zinc is less available to animals than organic zinc. Dietary supplementation with organic zinc sources like Bioplex Zinc (BZ) has been found to be beneficial for male ducks and pigs. Information on the dietary effects of the use of BZ in cocks has not been adequately documented. Therefore, growth, organs histopathology and semen characteristics of cocks fed dietary BZ were evaluated.

One-day-old Nera black cockerels (n=240) were allotted to four treatments containing 0, 100, 200 and 300 mg/kg of BZ in a completely randomised design with five replicates (n=12) at the starter, grower and finisher phases. The study lasted for 30 weeks to determine Daily Feed Intake (DFI), Daily Weight Gain (DWG), Feed Conversion Ratio (FCR) and Zinc Retention (ZR) using standard procedures. Blood (5 mL) was collected at 8, 16 and 24 weeks after treatment for leukocytes, glucose, cholesterol and triglycerides analyses using standard procedures. Semen characteristics, morphology of sperm cells, testicular morphometry and histopathology of testis, liver, kidney, ileum and spleen were assessed using standard procedures. Data were analysed using descriptive statistics, regression and ANOVA at $\propto_{0.05}$.

The DFI (174.5±1.4 g) and DWG (18.1±1.0 g) were significantly higher in birds on 300 mg/kg BZ at the finisher phase. The FCR (10.0±0.6 - 9.6±0.8) decreased with increasing levels of BZ. The ZR were 35.0±0.4, 44.0±0.1, 52.3±0.1 and 69.1±0.4 % for birds fed 0, 100, 200 and 300 mg/kg BZ respectively. Leukocyte counts ($10^{-3}/mm^3$) (14.7±0.01, 14.3±0.02 and 14.1±0.05) were significantly lowered in birds fed 100, 200 and 300 mg/kg respectively compared to the control (16.9±0.05). Serum glucose, cholesterol and triglycerides of cocks decreased with increasing BZ levels. Semen volume (mL) of birds fed 0, 100, 200 and 300 mg/kg BZ were 0.30±0.01, 0.30±0.004, 0.31±0.002 and 0.40±0.01 respectively and mass activity were not different among treatments. The birds fed 0, 100, 200 and 300 mg/kg of BZ recorded sperm motility of 77.0±0.1, 83.9±0.1, 84.6±0.3 and 88.1±0.4 % and live spermatozoa of 82.7±0.3, 86.7±0.3, 88.9±0.5 and 90.3±0.2 %

respectively. Sperm concentration (sperm cell/mL) (0.31±0.002, 0.33±0.003 and 0.45±0.001x10^9) respectively was higher in birds fed 100, 200 and 300 mg/kg of BZ than the control diet (0.29±0.008 x10^9).

Spermatozoa with rudimentary tails (%) (1.7±0.03, 1.4±0.02, 1.3±0.01 and 0.7±0.02) and curved mid-piece (%) (1.3±0.02, 1.2±0.01, 1.2±0.02 and 0.6±0.01) decreased as the BZ increased. The paired testicular weights (g) (29.4±4.7, 25.2±7.4 and 28.1±5.7), volume (cm^3) (25.8±8.2, 24.2±8.9 and 29.8±6.2) and density (g/cm^3) (2.3±0.4, 2.1±0.9 and 1.9±0.2) of birds fed 100, 200 and 300 mg/kg BZ were not different from the control (27.8±10.2, 26.4±8.6 and 2.1±1.0) respectively Histopathology of kidney from 300 mg/kg BZ showed renal congestions while testis, liver, spleen showed no visible lesions across treatments. The 81% of the variation in DFI was explained by inclusion levels of BZ.

Bioplex zinc increased growth rate, improved semen quality and reduced sperm cell abnormalities in cocks fed at 300 mg/kg.

Keywords: Bioplex zinc, Semen quality, Organ histopathology, Testicular morphometry.

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CERTIFICATION

We certify that this work was carried out by Mrs Olusola Olabisi **ADENEKAN** with Matriculation Number 129412 in the Animal Physiology and Bioclimatology Unit, Department of Animal Science, Faculty of Agriculture and Forestry, University of Ibadan, in partial fulfillment of the requirement for the award of degree of Doctor of Philosophy of the University of Ibadan under our supervisions.

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DEDICATION

To the Almighty God, the Omnipresent and Omniscience, who has been the Provider, Helper and Sustainer of my life throughout the duration of this path of success programme.

To my dear parents Chief and Madam M .O. Oloko for their continual support which gave me great encouragement and stability.

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Adenekan, Olusola Olabisi

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CHAPTER ONE

1.1 GENERAL INTRODUCTION

Global animal food production has been intensified and genetic potential for growth and yields has improved. Minerals are nutrients involved in a high number of metabolic pathways. Macroelements are expressed as a percentage of feeds or tissues, and are mostly involved in structural functions. Microelements, or trace minerals are required in very low concentrations in animal tissues. However, genetic advancements continually change the commercial broiler strains, and nutritionists start to question if currently used trace mineral levels and sources will be suitable in the future when feeding these faster-growing and highly productive birds for meat and eggs. The levels of inclusion of trace minerals in the feed are based mostly on NRC recommendations. Much of the information is actually based on research from the 1960s and 1970s during which the birds and their management were substantially different. Because of that, the NRC recommendations may not represent the needs of modern strains of commercial poultry, and commercial trace mineral inclusion levels often exceed NRC recommendations. As a result, commercial tendencies have been to increase trace mineral supplementation rates, in order to allow for the greater mineral requirements of superior stock reared under industrial conditions (Leeson, 2005).

Mineral utilization by animals primarily depends of their absorption from the ingested feed. In the feed, minerals are found in a wide range of chemical forms. They are found as organic molecules or as part of salts of varying solubility. Supplementary sources of organic minerals in the market are still recent; however, they brought attention to a group of nutrients that have been set aside for many years as less important. The interest inorganic forms include differences in availability, but also it is also related to possible improvements of their specific actions at cell level (Vieira, 2008) Inorganic and organic

mineral forms are found in animal tissues, both in variable concentrations. Inorganic elements are found in the ash left after combustion of live tissues mostly as oxides, carbonates, and sulfates (Underwood and Suttle, 1999). Out of the 109 known elements, 26 are considered essential for animals. From these, 11 are macro elements (carbon, hydrogen, oxygen, nitrogen, sulfur, calcium, phosphorus, potassium, sodium, chlorine, and magnesium) and 15 are microelements (iron, zinc, copper, manganese, nickel, cobalt, molybdenum, selenium, chromium, iodine, fluorine, tin, silicon, vanadium, and arsenic) (Vieira, 2008). The macro-minerals are required in amounts greater than 100 mg/dl and the micro-minerals are required in amounts less than 100 mg/dl (Murray *et al*., 2000).

Supplementary inorganic trace minerals have traditionally provided poultry diets with sufficient amounts of each mineral to support normal growth, health, and reproduction (McCartney, 2008). Since 1950s, animal feeds have been supplemented with essential trace minerals such as cobalt (Co), copper (Cu), iron (Fe), iodine (I), manganese (Mn), molybdenum (Mo), selenium (Se) and zinc (Zn). Traditionally, inorganic salts such as chlorides, oxides, sulphates and carbonates have been added to the diet to provide the desired amount to meet the requirements of the animal (Mc Cartney, 2008). These are broken down to varying extent during digestion to 'free' ions and are then absorbed. However, they may also complex with other dietary molecules and become difficult to absorb or, if completely complexed, totally unavailable to the animal. Thus, the availability of the element may vary substantially. Because of these uncertainties, the levels provided in the diet are often higher than the minimum required for optimum performance, often resulting in over supply and unnecessary wastage with obvious environmental impact (Boland, 2003).

Trace mineral chelates have proven to be better than inorganic minerals in meeting the nutritional needs of modern farm animals. Chelates are organic forms of essential minerals such as copper, iron, manganese and zinc. Animals absorb, digest and use mineral chelates better than inorganic minerals. These organic mineral proteinates are defined as a mineral that is bound to an amino acid or peptide and are more bio-available than inorganic salts for three main reasons: The structure protects the mineral from

unwanted chemical reactions in the gut; they are absorbed more efficiently in the gut and utilised by physiological processes; and because they are not charged, there are fewer negative mineral interactions This means that lower concentrations can be used in animal feeds. In addition, animals fed chelated sources of essential trace minerals excrete lower amounts in their faeces and so there is less environmental contamination. Mineral chelates also offer health and welfare benefits in animal nutrition (McCartney, 2008). Compared to inorganic sources, chelates of Zn, Cu and Mn have been shown to confer stability in the upper digestive tract, thereby avoiding binding with antagonists and improving bioavailability (Leeson, 2005; Bao and Choct, 2009)

Birds naturally obtain their minerals from the leaves and grains they ingest. In plants, these minerals are bound to small peptides. In this form they are preferentially taken up in the gut via the same systems that absorb amino acids. In contrast, inorganic minerals are far less bio-available and are excreted at a much higher rate. Increasing concerns within the European Union regarding the level of soil and water pollution from intensive agriculture have led to discussions and restrictions regarding the supplementation of poultry diets with inorganic minerals. The use of lower levels of minerals of organic Bioplex has clearly demonstrated that performance can be maintained, while improving other aspects of health and welfare (Steveson, 2009).

Zinc is widely distributed throughout the body and plays an essential role in many body processes: for the development of physiological functions such as feathering, bone development and immunity. Organic zinc minerals have the potential benefit of improving mineral utilisation while reducing their excretion (Steveson, 2009). Zinc is present in many enzyme systems that are concerned with the metabolism of feed constituents. For example, zinc is a constituent of carbonic anhydrase, carboxypeptidase A and B, several dehydrogenases, alkaline phosphatase, Ribonuclease and DNA polymerase (Underwood and Shuttle, 1999).

Zinc is required for normal protein synthesis and metabolism, and it is also a component of insulin so that it functions in carbohydrate metabolism. Because zinc plays so many

important roles in the body, therefore it is required by all livestock and poultry (Miles and Henry, 2000). National Research Council (1994) recommended a level of 40-75 ppm zinc in various poultry diets while the studies of (Mohanna and Nys, 1999; Huang *et al*., 2007; Linares *et al*., 2007) indicate a requirement of 40–50 mg kg−1 DM for optimum growth up to 21 days of age in chicks' growth. Huang *et al*. (2007) concluded that 84 mg Zn kg−1 DM was optimal, but the justification for these as 'preferred indices' of need was unconvincing. The mineral tolerance of zinc level in the poultry diets at which point adverse physiological effects are observed is 800-4000mg/kg (Larry, 1987) while NRC (1980) gave a mineral tolerance level of 1,000ppm to swine, turkeys and chickens fed nonpurified diets.

Cockerels are male fowls that are less than a year old (Poultry study guide, (2004). Cockerels produced in most developed countries are destroyed and in developing countries like Nigeria, they are sold at a giveaway price since the primary concern of most hatcheries is the production of commercial pullets and broilers. This constitutes a remarkable loss to the hatchery industry. Cockerel rearing has attracted considerable interest among poultry farmers. A survey by Esiemokhai (1986) showed that consumers prefer cockerel meat because it is not as tender as other poultry meat. They also claimed that it is more palatable. Cockerels are mostly raised under the extensive system because they are hardy, raised on bulk feeds of relatively low nutrients values mainly kitchen waste as compared to broilers and layers raised on high energy rations. Scientific investigations on the nutrition of poultry has conspicuously neglected cockerel as a scientific class of poultry. Little attention has been paid to commercial cockerel production in Nigeria. This is due to the fact that cockerels are light breeds with slow growth rate and are poor feed converters (Oluyemi and Roberts, 1979).

The present study was focused on the growth, histopathology of organs and semen characterstics of cocks fed different dietary levels of Bioplex zinc

1.2 JUSTIFICATION OF STUDY

Although there is abundance of scientific data to show the growth enhancing potential of zinc in livestock production, little has been reported on its influence on the reproductive efficiency of cocks.

The bioavailability of organic zinc enhances nutrient absorption and utilization hence reduces environmental pollution. Bioplex zinc is needed in lower concentration without compromising the optimum performance, productivity of the animals and profitability of the farmers (Stevenson, 2009).

There is therefore the need to study the effect of the inclusion of Bioplex zinc on the semen characteristics of such animals. Also this work will broaden the knowledge of the use of zinc in animal nutrition especially poultry and look at its effect on the performance, blood composition, serum biochemistry, carcass characteristics and the histopathological examination of the cocks.

1.3 OBJECTIVES OF STUDY

The present study is therefore designed to determine the effects of dietary Bioplex zinc (a chelated zinc proteinate) produced by Alltech Inc Kentucky USA in July 2010, Batch No: 9791- (1-10) on the:

1. Growth performance and nutrient digestibility of cockerels
2. Haematology, serum biochemistry of cocks.
3. Carcass and Organoleptic characteristics, relative organ weights and the histopathological examinations of some selected organs of cocks.
4. Zinc retention levels in the body tissues of cocks.
5. Semen characteristics, sperm morphology, testicular and epididymal morphometry of cock

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION: ZINC

The first unequivocal evidence that zinc is necessary for growth and health was obtained in laboratory animals (Todd *et al*., 1934) at a time when associations were being noted between zinc and carbonic anhydrase, an enzyme eventually shown to contain zinc (Keilin and Mann, 1940). In domesticated species, experimental zinc deprivation was produced first in chicks (O'Dell and Savage, 1957) and pigs (Stevenson and Earle, 1956) and later in lambs (Ott *et al*., 1964) and calves (Mills *et al*., 1967). Zinc is a micro-mineral involved in various processes of animal metabolism. Since it was originally demonstrated that zinc is necessary for healthy growth of rats (Tood *et al.*, 1934), the role of zinc in the animal organisms began to gain special attention (Suttle, 2010)

2.1.1 HISTORY

According to International Zinc Association (2011), in 1934 zinc was discovered in the metallic form in India as a new metal – the 8th metal known to man at that time. At Zawar, India, both zinc metal and zinc oxide were produced from the 12th to the 16th century. Zinc metal was used to make brass, and zinc oxide served medical purposes. From India, zinc manufacturing moved to China in the 17th century where it developed as an industry to supply the needs of the brass industry.

Zinc was recognized in Europe as a separate metal in the 16th century when Agricola (1490–1555) observed when a metal called "zincum", produced in Slesia and Paracelsus (1493–1541), stated clearly that "zincum" was a new metal. Its ores were used for making brass and zinc compounds and also for healing wounds and sore eyes. It is believed that the Romans first made brass in the time of Augustus (20 B.C–14 A.D.). In the 13th century Marco Polo described the manufacture of zinc oxide in Persia. In 1743, the first European zinc smelter was established in Bristol in the United Kingdom using a vertical

retort procedure. Pure metallic zinc was discovered by Andreas Sigismund Marggraf in 1746 in Germany. Origin of name: from the German word "zink". A major technological improvement was achieved with the development of the horizontal retort process in Germany which led to the erection of smelting works in Slesia, Liege, Belgium and Aachen, the Rhineland and the Ruhr areas in Germany. In 1836 hot-dip galvanizing, the oldest anti-corrosion process was introduced in France. Zinc production in the United States started in 1850.

Zinc has been used by man for industrial, ornamental or utilitarian purposes for 2000 years. It has been alleged that Zn was applied as an ointment for skin lesions by several cultures of the ancient world, including the Egyptians. For over 100 years Zn has been known to be needed for the growth of the fungus Aspergillus Niger. Later, the presence of Zn numerous plant and animal tissues was established. The nutritional essentiality of Zn was demonstrated first in 1934 by Bertrand and Bhattacherjee in mouse, during the same year by Todd and co-workers in the rat as reported (Underwood, 1977).

The discovery that Zn prevents and cures parakeratosis in pigs and that this disease can occur on commercial-type diets in the presence of excess calcium (Ca) gave a great stimulus to the studies of the nutritional physiology of Zn in farm animals. O'Dell *et al*., (1958) showed that Zn deficiency was responsible for the poor growth and abnormal bone development in chicks receiving purified diets. Based on the Zn requirement of ruminants using purified diets, it was generally assumed that herbage grown on Zn –deficient soils carried enough Zn for the needs of these animals. The assumption was proved invalid, when Legg and Sears (1974) first demonstrated that parakeratosis-type skin disorder in grazing cattle of Guyana responded to Zn therapy.

For about 500 years zinc was produced from its oxide ores before the more abundant sulfides became the major source of supply. On the technological side, there was a drastic change in 1916 when the electrolytic process was introduced on a large scale replacing the pyrometallurgical process as the dominating production method (International Zinc Association, 2011).

2.1.2 CHEMICAL PROPERTIES

Zinc is a bluish white, relatively soft metal with a density slightly less than that of Fe (7.133 and 7.86 g/cc respectively). Zinc is a divalent cation, with Symbol: Zn, an atomic number of 30, atomic weight of 65.37, Group in periodic table: 12, Period in periodic table: 4, Block in periodic table: d-block, Classification: Metallic and is found in ores principally as the sulfide (ZnS). It is often found associated with sulfides of the other metals, especially lead (Pb), copper (Cu), cadmium (Cd), and iron (Fe). Zinc is twenty-fourth in abundance of the chemical elements (International Zinc Association, 2011).

2.2 ZINC IN ANIMAL TISSUES AND FLUIDS

2.2.1 GENERAL DISTRIBUTION

Species differences are clearly small and zinc occurs widely in relatively high concentration throughout the body. The mammalian newborn does not consistently carry higher total body Zn concentrations than mature animals of the same species and there is little fetal Zn storage. During the suckling period whole body Zn concentration rises substantially from newborn levels in the rat and pig but not in the guinea pig. The whole body of adult man is estimated to contain 1.4–2.3g of Zinc, of which about 20% is present in the skin. The mean zinc concentrations of normal human epidermis and dermis have been reported to be 70.5 and 12.6pg/g dry weight, respectively (Hambidge *et al*., 1986).

Zinc occurs in the earth's crust to the extent of about 70-130mg/kg. Normal soils contain 10-300mg/kg Zn, average about 50mg/kg, and in uncontaminated areas, the contents generally are not very different from those of the parent rock (NRC, 1994). Soils near highways can be contaminated appreciably with the Zn from tires (containing Zn oxide) and emissions from motor oil to which Zn dithiophosphite has been added. Zinc values (dry weight) for forages range between 17 and 60 mg/kg; cereal grains, 20-30mg/kg; oilseed meals, 50-70mg/kg, and animal protein sources, 90-100 mg/kg. A drinking water standard is 5mg/kg; with industrial pollution greatly increasing Zn in both water and plant sources (NRC, 1994).

The normal human body contains about 2.2g Zn, an overall concentration of about 30mg/kg. Similar concentrations of Zn occur in most other animals. In contrast to most trace elements, Zn is fairly evenly distributed throughout the tissues of animals. With the

exception of some specialized tissues that may contain much higher levels, the concentrations of Zn in most mammalian tissues are in the order of 10 to 100 µg/g wet weight (30-250 µg/g dry weight), with little variation among species. The highest concentrations are found in the epidermal tissues, such as skin, hair, feathers, and wool. In liver and mammary cells the zinc is present in the nuclear, mitochondrial and supernatant fractions, with the highest levels per unit of protein in the supernatant and microsomes. Experiments with ^{65} Zn injections in mice indicate that about one-sixth of the ^{65} Zn in the tissues is firmly bound to protein and cannot be removed by dialysis or by ethylenediaminetetraacetic acid (EDTA). This fraction probably consists of Zn metalloenzymes and the Zn bound to nucleic acids. The remainder which is exchangeable with Zn ions or removable by EDTA is bound to the imidazole or sulfhydryl group of the proteins (Hambidge *et al*., 1986).

The Zn concentration of the tissues, apart from the blood, milk and hair, do not always reflect dietary Zn intakes. Under deficiency conditions the growth of the organs can be so inhibited that while the total amount of zinc is greatly reduced, the concentrations may not be significantly lowered. The heart and the testes remain relatively insensitive to very high-Zn intakes, but large increases in the Zn concentrations of the plasma, liver, kidney and spleen have been demonstrated in rats fed a normal diet plus 1000 and 2000mg/kg of supplementary Zn for 15 days (Underwood, 1981).

2.2.2 MINERAL BIOAVAILABILITY

Mineral bioavailability is defined as the proportion of the element consumed that is utilized for a biochemical or physiological function (O'Dell, 1997). For a mineral to have high bioavailability it must be readily absorbed and easily assimilated by the body. Consequently, bioavailability is influenced by the chemical form of the mineral, the amount in the diet, the amount stored in the body, the concentration of other minerals in the diet, and the health, age, and physiological state of the animal to which it is fed (O'Dell, 1997; Miles and Henry, 2000).

The presence of a mineral in animal tissues does not imply its essentiality. Minerals can be natural contaminants and, in this case, they follow a similar distribution as that found in the surrounding environment. Essential elements, however, exist in a normal and expected symmetry, and their deficiency results in impairment or loss of organic

functions (Underwood, 1981). The presence of essential elements in animal tissues follows cell function and, therefore, they have typical concentrations for each organ. As occurs with any other nutrients, minerals can cause toxicity if ingested in high levels or for long periods. The biological availability of a mineral from the diet is manifested by the efficiency with which the body utilises and retains the dietary mineral. The retention will be influenced by a number of dietary factors, including diet or ingredient type, source of minerals, levels and relative proportions of various minerals (Thomas and Ravindran 2010).

2.2.3 ZINC IN MALE SEX ORGANS AND SECRETIONS

The Zn concentrations of the testes of rats ranging in age from 7 to 58 days remains fairly constant at about 120 mg/kg dry weight for the first 30-35 days and then increases to close to 20mg/kg during the second month of life, when spermatids are transformed into spermatozoa. Values of 176 ± 12 and 132 ± 16 mg/kg Zn (d.b.) for the testes of normal and Zn-deficient rams (Underwood, 1981) have been reported. Lower mean levels of 17 and 13µg Zn/g wet weight have been found for the normal adult human testis. The values are close to 85 and 65mg/kg on the dry basis.

The level of Zn in the prostate is reduced by castration, and the rate of Zn accumulation is greatly increased in young rats by testosterone or gonadotropin injections. Chronic gonadotropin increases the weight and ^{65}Zn uptake in all the other accessory reproductive structures in male rat as well as the dorsolateral prostate, whereas FSH (follicle stimulating hormone) reduces this uptake per organ and gram of tissue. The administration of stilbestrol lowers the Zn content of human prostatic cancerous tissue, which even before treatment is lower in Zn concentration than that of normal, or hyperplasic but non malignant prostatic tissue (Underwood, 1981).

2.2.4 ZINC IN BLOOD

Zn is present in the plasma, erythrocytes, leukocytes and platelets, 30–40% of the plasma Zn is firmly bound to a α_2–macroglobulin, and 60–70% is loosely bound to albumin Almost all the Zn in erythrocytes occurs as carbonic anhydrase, together with the small fraction associated with other Zn enzymes. Carbonic anhydrase cannot be detected in plasma or leukocytes, so that the Zn in erythrocytes must account for all these enzymes in the blood. In newborn infants the Zn content of the erythrocytes is only one-quarter of the

adult value, rising progressively over the first 12 years of life. This would be expected from the low levels of carbonic anhydrase present in the red cells of newborn and premature infants.

The following normal levels for Zn in erthrocytes were reported; man, 13; rat, 10; dog and rabbit, 9; and goose, 6.5µg/g. in the adult rabbit, average Zn level are 2.5µg/ml of whole blood, 2.7µg/ml of plasma, and 9µg/ml of erythrocytes. In young growing pigs 0.6µg Zn/ml of plasma and 7µg/ml of erythrocytes can be considered normal (Underwood, 1981). Profound changes occur in the levels of Zn in the blood plasma and cellular elements in various disease states and under stress conditions. Serum Zn concentrations were shown to decrease significantly in a dose- dependent response after endotoxin administration in the rat. These abnormalities in Zn metabolism manifested in depressed serum Zn levels are little understood. However, Beisel *et al*. (1974) observed a redistribution of Zn within the body initiated by a hormone-like protein factor, which is release from phagocytizing cells. This factor, leukocytic endogenous mediator (LEM), stimulates the liver to take up Zn (and lron). In patients with anemia, other than pernicious anemia, the Zn and carbonic anhydrase levels in the blood are lowered in parallel fashion with the decreases in hemoglobin and red cell counts, but in pernicious anemia the Zn and carbonic anhydrase per unit of RBC are significantly above normal, even when the increase cell size is eliminated as a contributing factor.

2.2.5 ZINC IN THE EYE TISSUES

Zinc maintains normal concentrations of vitamin A in plasma and is necessary for the normal functioning of the general epithelium of the ovary. These workers used animals deficient in both Zn and vitamin A and demonstrated that synthesis of the retinol-binding protein (RBP), the carrier of vitamin A in the blood, is decreased in Zn deficiency, resulting in inadequate vitamin A mobilization from the liver. The precise mechanism involved is not clear. Thymidine kinase and DNA-dependent RNA polymerase depend on Zn for their activity and are vital to protein synthesis. They could, therefore, be concerned in RBP synthesis (Underwood, 1981). Activity of alcohol dehydrogenase is depressed in the liver of Zn-deficient lambs, and could be related to the night-blindness observed in some lambs. A postulated Zn metalloenzyme is an alcohol dehydrogenase, necessary for the interconversion of vitamin A alcohol (retinol) to vitamin A aldehyde (retinal), a

process essential for normal vision. Vitamin A and zinc are powerful antioxidants with synergy between them, thus protecting the organism against oxidative stress during the pre and postoperative periods (Underwood, 1981). Vitamins A and E metabolism and bioavailability are dependent on zinc status (Szabo et al., 1999). The highest Zinc concentrations known to exist normally in living tissues occur in the choroids of the eye and optic nerve. Eye tissues other than the choroids and the iris carry more normal Zn concentrations. The functions of zinc in such high concentrations in eye tissues are still under investigations (David and Watts, 1988).

2.2.6 ZINC IN THE AVIAN EGG

The mean concentration of Zn in egg contents of domestic chicken 34.22 ±3.4 µg g^{-1}, quinea fowl 34.20±7.4 µg g^{-1}, pigeon 25.29±3.06 µg g^{-1} and quail 48.47±11.6 µg g^{-1} respectively has been reported by Abdujaleel and Shuhaim-Otham (2011). Most of the Zinc is present in the yolk associated with the lipoprotein and lipovitellin. Lower levels of zinc than those just given occur in eggs from hens on Zn-deficient diets.

2.3 ZINC METABOLISM

ABSORPTION: In rats, Zn is absolved mainly from the duodenum, ileum and jejunum, with very little being absorbed from the stomach or colon. In cattle about one third of an oral dose of ^{65} Zn was apparently absorbed from the abomasums, with further absorption occurring throughout the small intestine. In calves, Zn absorption also occurs throughout the small intestine, with the amount absorbed per unit of length being as great in the distal as in the proximal ends. Substantial Zn absorption from the proventriculus, as well from the small intestine, occurs in chicks.

The mechanism of Zn absorption and its control has been illuminated by the work of Evans and his associates. They have described a low molecular weight Zn–binding factor in the intestinal lumen, intestinal mucosa, and pancreas of rats and pancreatic secretions from a dog (Evans *et al.*, 1975). The uptake of ^{65} Zn by epithelial cells from averted intestinal rat segments was found to be markedly increased in the presence of this Zn-binding ligand from pancreatic secretion, and 30% of the epithelial cell was associated with the partially purified basolateral plasma membrane. When these membranes were incubated in a medium that contained Zn-free albumin, some 96% of the ^{65} Zn was transferred to the medium, while less than 30% was released to media that contained

either no albumin or a 3:1 Zn-albumin complex. These findings led Evans *et al.* (1975) to propose the following sequence for Zn absorption:

a. The pancreas secretes a Zn-binding ligand into the intestinal lumen
b. ln the lumen Zn binds to the ligand
c. Complexed with a ligand, Zn is transported through the intestinal microvillus and into the epithelial cells.
d. In the epithelial cell Zn is transferred to binding sites on the basolateral plasma membrane.
e Metal-free albumin interacts with the plasma membrane and removes Zn from the receptor sites.

The quantity of metal-free albumin available at the basolateral plasma membrane, it is contended, determines the amount of Zn removed from the intestinal epithelial cell and thus regulates the quantity of Zn entering the body. Whether this can be regarded as a fully acceptable hypothesis for the mechanism controlling Zn absorption will only be known as research proceeds. Dietary Zn is known to be sequestered into mucosal cell Zn-binding proteins formed in response to Zn, thus inhibiting its transfer of serum albumin and permitting the excretion of sequestered Zn via the desquamations of mucosal epithelium. Copper and the metals inhibiting Zn absorption do so at least in part by competing with Zn for binding sites on the ligand or ligands from the pancreatic secretions in the intestinal lumen provide a further opportunity for those metals to inhibit Zn absorption. The extent of Zn absorption varies with the level of Zn and with a range of other dietary components.

High inorganic P as well as high-Ca intakes aggravates Zn deficiency in rats, and their effects appear to be additive and independent. An independent action of phosphorus on Zn absorption remains to be clearly established. The chemical form in which the zinc is ingested also influences absorption, so that diets of similar Zn contents may differ in the amounts available to the animal. Different protein sources vary in their effects on the Zn requirements of rats, pigs, chicks and poults, with diets containing protein of plant seed origin requiring higher levels of dietary Zn than those containing protein from animal sources, O'Dell (1997) attributed such differences to the presence or absence of phytates which bind the Zn in a form from which it is not readily released and absorbed. The

addition of phytic acid to casein diets can reduces Zn retention to that in soybean protein diets, and Zn absorption from combinations with phytic acid is increased by autoclaving or by treatment with EDTA. The presence of phytate is only one of the factors effecting Zn availability from oilseed meals. Various chelating agents can improve the availability of Zn to chicks and poults consuming soybean protein diets. Furthermore, certain natural feeds such as casein and liver extract contain chelates, which improve Zn absorption and utilization. Many salts occur in nature as proteinates or chelates. Chelates may utilize peptide or amino acid uptake pathways rather than normal mineral ion uptake pathways in the small intestine. This prevents competition between minerals for the same uptake mechanisms. Not only is bioavailability therefore higher, but these mineral forms are more readily transported and hence their intestinal absorption is also enhanced. They are more stable and are protected biochemically from the adverse reactions with other dietary nutrients that could reduce their rate of absorption. It is also thought that they can be specifically targeted at certain organs, tissues or functions in the body (Boland, 2003).

Vitamin D had been reported to increase Zn absorption. The increased Zn absorption attributed to vitamin D is not a direct effect of the vitamin but results from a homeostatic response to the increased need for Zn which accompanies stimulated skeletal calcification and growth. From the work of Evans *et al*. (1975) it seems that the inhibition by copper of Zn absorption takes place at the site of transference from the mucosal cells to the plasma, since they demonstrated Cu-inhibited ^{65}Zn absorption in rats without affecting intestinal uptake of the isotope. Mention has already been made of the depressing effect of Copper on Zn absorption. This was shown using isolated duodenal segments of the rats. This worker had previously shown that high–Zn intakes depress Cu absorption conversely; Cu absorption is greatly increased in Zn deficiency. A mutual antagonism between Zn and Cu at the absorptive level is thus apparent (Hill *et al*., 2000).

2.4 STORAGE

Zinc is widely distributed throughout the body; however, animals have a limited capacity for storing Zn in a form that can be mobilized rapidly to prevent deficiency (Underwood, 1977). In general, readily available stores of Zn are quite small, as dramatically reflected by decreases in the plasma Zn values to the deficiency range within 24 hr after changing to diets very low in Zn. Metallothionein acts as a major storage form of Zn in the liver

and are mobilized during metabolic need. The superoxide dismutase fraction from liver is suggested also as a storage form of Zn (Underwood, 1981).

Studies of cattle with "Zn indicate that the highest concentration was found in the soft tissues in the order of pancreas, liver, pituitary gland, kidney, and adrenal gland. Other reports have shown that the testicles and accessory sex glands of the male contain high concentration; the digestive tract secretions also were relatively high. Primarily, Zn is associated with protein and skeletal tissues, with little found in the lipid portion. One biochemical characteristic of Zn is its ability to form complexes with the side chains of proteins. When young ruminants are fed a very Zn-deficient diet, the Zn content of some tissues declines, but in others, there is little or no change. With a severe deficiency, there is limited Zn reduction in hair, bone, liver, lung, kidney, spleen, pancreas, and blood plasma (Underwood and Suttle 1999).

2.5 EXCRETION

Zn leaves the body largely by the way of feaces. Feacal Zn consists mostly of unabsorbed dietary Zn with a small amount of endogenous origin secreted into the small intestine. In the calf the pancreatic juice contributes about one–fourth of the total endogenous Zn loss. Small amounts are also secreted into the bile, ceacum and colon. Injected Zn is similarly excreted mostly in the feaces. Radiozinc administered orally to steers was recovered 70% in the feaces and 0.3% in the urine. When the Zn was given intravenously, 20% appeared in the feaces and 0.25% in the urine. This pattern of excretion was followed on normal diets and on diets high in Zn. Endogenous excretion of ^{65} Zn and stable Zn is significantly reduced in calves and goat on-low-Zn diet, thus contributing to the homeostatic control of this element achieved primarily through increased absorption (Robinson *et al.,* 1973).

The quantity of Zinc excreted in the urine of healthy human adult is small (0.3-0.6mg/day), compared with the 10–15mg/day normally ingested. The amounts so excreted do not vary greatly with dietary Zn levels and are not significantly increased following Zn injection. Urinary Zn excretion is well above normal in nephrosis, postalcoholic hepatic cirrhosis and hepatic porphyria Six patients with albuminuria averaged 2.1mg Zn/day in their urine (range 1.0-3.8), whereas healthy individuals excreted only 0.3mg Zn/day. These heavy losses can be explained by the inability of the nephritic kidney to provide an effective barrier against the protein–bound Zn of the

plasma. The pronounced zincuria of postalcoholic hepatic cirrhosis is less easily understood. Accumulative zincuria has also been observed following major operations and severe burns, its severity and duration being commensurate with the metabolic insult or injury incurred. Increased urinary Zn excretion occurs in total starvation in man and during the administration of EDTA and DTPA (diethylenetriaminepenta acetic acid) (Robinson *et al*., 1973). The oxide, carbonate, and sulfate forms of zinc are efficiently utilized, whereas the sulfide form is poorly utilized. Zn, either ingested or injected, is primarily excreted in the faeces. The Zn found in the faeces consist mainly of unabsorbed dietary Zn, and the balance is from pancreatic excretions. Urinary excretion of Zn and several other metals are increased if chelating agents such as ethylenediaminetetraacetic acid (EDTA) are administered in combination with zinc (Hays and Swenson, 1985).

2.6 INTERMEDIARY METABOLISM

The Zn absorbed from the intestine is carried to the liver in the portal plasma bound to transferin. In the venous plasma the Zn is mostly bound to albumin and to a small extent to transferrin and α_2– macroglobulins. This Zn is incorporated at differing rates into different tissues, which reveal varying rates of Zn turnover, Zn uptake by the bones and the CNS is relatively slow and remains firmly bound for long periods. The Zn entering the hair is not available to tissue and is only lost as the hair is shed (Evans *et al*., 1975)

The Zn in bones is also not normally readily available for metabolic use. The most rapid accumulation and turnover of retained Zn occur in the pancreas, liver, kidney and spleen. In the rat radiozinc accumulates most rapidly in the pancreas and dorsolateral prostate. The Zn in these tissues, and the more slowly exchanging muscle and red cell Zn, constitute a soft tissue Zn pool composed of compartments of varying exchange rates. The major organ involved in Zn metabolism is the liver. The metabolic role of metallothionein, in addition to its role in cellular metal detoxification mechanisms, is to serve as a storage protein for Zn, analogous to ferritin for Iron (Underwood, 1981).

2.7 PHYSIOLOGICAL FUNCTIONS

2.7.1 EFFECT ON GROWTH, APPETITE AND TASTE

Zinc is required for the structural and functional integrity of over 2000 transcription factors and almost every signaling and metabolic pathway is dependent on one or more

zinc-requiring proteins (Beattie and Kwun, 2004; Cousins *et al*., 2006) Growth retardation is universally observed in Zn deficiency, perhaps because of impairment of nucleic acid biosynthesis (O'Dell, 1997). It probably arises primarily from a decreased activity of thymidine kinase and hence impaired DNA synthesis and cell division. Zinc deficiency also results in impaired amino acid utilization or protein synthesis. Zinc is needed for functions of the taste buds (Merck, 1986; Murray *et al.,* 2000). Loss of appetite is one of the first signs of deficiency; with poor growth, it may be the only overt sign of a mild deficiency. Severely Zn-restricted rats eat as little as one third that of ad libitum-fed controls, with significantly less fluid. Reduced feed intake may relate to the role of Zn in taste Subsequent to the onset of deficiency and anorexia, there is loss of taste acuity (Hambidge *et al.,* 1986).

Zinc deprivation increases the expression of the gene for the appetite-regulating hormone cholecystokinin (Cousins *et al*., 2003), but the role of zinc is probably multifactorial. There is also increased expression of leptin, a cytokine hormone whose secretion from adipocytes acts as a satiety signal (Kwun *et al*., 2007), and reduced expression of pyruvate kinase (Beattie *et al*., 2008), which is highly regulated by insulin. The growth inhibition of Zn deficiency results partly from impaired appetite, i.e., reduced food consumption, and partly from impaired food utilization. It was found that the voluntary food intake of Zn-deficient rats to fall to 70% of the controls, with marked day-to-day variation in intake and a cyclical pattern of food consumption. The Zn-deficient rats responded to a Zn-supplemented diet with 1-2 hrs by an increased food intake. A physiological role for Zn in normal taste sensation was first established by Henkin *et al.* (1971), together with the further important observation that the hypogeusia (loss of taste acuity) and dysgeusia (disordered taste or pica) that commonly occur in adults can respond to oral Zn therapy. Depletion of total body Zn by oral administration of histidine also produced anorexia and hypogeusia in man and was reversed by Zn administration.

2.7.2 REPRODUCTION:

Spermatogenesis and the development of the primary and secondary sex organs in the male and all phases of the reproductive process in the female from estrus to parturition and lactation can be adversely affected in Zn deficiency. Zinc is required for normal testicular development (Merck, 1986). Atrophic seminiferous tubules, retarded development of the testes, epididymis, prostate and pituitary glands were observed in the

earliest study of the histopathology of Zn deficiency in the rat. The testicular atrophy and failure of spermatogenesis, on the other hand, is due directly to lack of Zn. The availability of sufficient Zn for incorporation of high amounts into sperm during the final stage of maturation is essential for the maintenance of spermatogenesis and survival of the germinal epithelium. Impaired development and functioning of the male gonads are apparent in other species. Hypogonadism, with suppression of the secondary sexual characteristics, is a conspicuous feature of the conditioned Zn deficiency (Underwood, 1981).

In the experiment with lambs testicular growth was greatly impaired and spermatogenesis ceased within 20-24 weeks in a diet containing 2.4mg/kg Zn, whereas no such effects were observed in comparable pair-fed lambs on an intake of 32.4mg/kg Zn. The body growth and food consumption of the two groups of lambs receiving unrestricted diets containing 17.4 and 32.4mg/kg Zn were similar, but testicular growth and sperm production were significantly greater in the rams receiving the larger Zn supplement (Underwood, 1981). These differences wer e further reflected in the histological ratings given to the testes of the animals from the different treatments. The fructose concentration of the seminal plasma, which provides an index of testosterone production by the gonads was similar for all dietary treatments.

2.7.3 SKELETAL DEVELOPMENT

Skeletal abnormalities are a prominent feature of Zn deficiency. In poultry, long bones are shortened and thickened; in calves, a bowing of the hind legs and stiffness of joints is noted. Chick bones show reduced epiphyseal cartilage width and less cell division. Bone collagen synthesis and turnover are markedly reduced, with reduced activity of tibial collagenase, a Zn metalloenzyme. Skeletal abnormalities are a prominent feature of Zn deficiency in growing birds. This has been demonstrated in chicks, poults, pheasants and quail. The long bones are shortened and thickened in proportion to the degree of Zn deficiency. Changes and disproportions occur in other bones, giving rise to a perosis histological similar to that of Mn deficiency (O'Dell 1997; Murray *et al*., 2000). Gross skeletal and other deformities including ageness of the limbs, dorsal curvature of the spine, and shortened and fused vertebrae develop in chick embryos from the eggs of hen fed severely Zn-deficient diets. Leg defects do not develop in Zn-deficient chicks on all types of diets, even when other signs of the deficiency are apparent. These "perosis-like"

or “arthritis-like” abnormalities can be alleviated by histamine, histidine and various antiarthritic agents without affecting the other manifestations of deficiency. Supplementary Zinc prevents both the leg disorder and the other signs of Zn deficiency (Underwood, 1981; Leeson and Summers, 2001). Higher dietary levels of Zn are required in the presence of phytic acid to prevent parakeratosis and allow for normal growth (Sidhu *et al*., 2004).

2.7.4 KERATOGENESIS:

Keratin is the major structural protein of the hoof horn, feathers, skin, beaks and claws, while collagen is the major structural protein of the extracellular matrix and connective tissues in internal tissues, including cartilage and bone. Decreases in collagen and keratin synthesis rates in zinc-deficiency can lead to a variety of defects including bone abnormalities, poor feathering, decreased tissue strength and dermatitis (Underwood and Suttle, 1999; Leeson and Summers, 2001). Alopecia and gross skin lesions were observed in the early investigations of Zn deficiency in rats and mice. Histological studies disclosed a condition of parakeratosis, i.e. thickening or hyper keratinization, with failure of complete nuclear degeneration, of the epithelial cells of the skin and eosophagus. In more severe Zn deficiency, scaling and cracking of the paws with deep fissues develop, in addition to loss of hair and dermatitis. In pigs, parakeratosis mostly occurs around the eyes and mouth and on the scrotum and lower parts of the legs. A similar distribution occurs in Zn-deficient ruminants, with the legs becoming tender, easily injured and often raw and bleeding (Underwood, 1981). Experiments in poultry have demonstrated that bone breaking strength correlates strongly with the extent of collagen crosslinking (Rath *et al*., 1999).

In the Zn-deficient chick, poult and pheasant, feathering is poor and abnormal and dermatitis is usual. In the Japanese quail, feathering is severely affected, due to a degeneration of the feather follicles resulting from hyperkeratosis. Involvement of Zn in keratogenesis is further evident from the gross disturbances of the integument, as well as the skeleton, observed in chick embryos from the eggs of severely Zn-deficient hens. Acrodermatitis enteropathica, a hereditary disease appearing in early infancy characterized by pustular and eczematoid skin lesions, alopecia and diarrhea, is due to aberrant Zn metabolism and responds to Zn therapy (O’Dell 1997).

2.7.5 WOUND HEALING

Zinc is needed for tissue repair and wound healing (Murray *et al.,* 2000). The first indications of a role for Zn in wound healing came from the studies of Pories *et al.* (1968), who demonstrated a significantly increased rate of wound healing, compared with unmediated controls, when zinc sulfate, at the rate of 50mg Zn three times daily, was added to the diet of young men following surgery for pilonoial sinuses. The enhancement of wound healing by Zn may stem from a heightened metabolic demand for this element for collagen synthesis in the process of tissue repair, with an increase in collagen synthesis and cross-linking explaining gains in wound tensile strength (McClain *et al.*, 1984), but direct evidence for this is lacking. Furthermore, Zn responsive differences in tissue repair could be related to differences in the rate of cell division and DNA production in rapidly regenerating tissue, since Prasad *et al.* (1975) have shown that depressed activity of thymidine kinase for DNA synthesis and cell division is an early metabolic defect on Zn deficiency.

2.7.6 ATHEROSCLEROSIS

Indications have been obtained that Zn can be beneficial in some cases of atherosclerosis. Atherosclerosis is a multifactorial disease to which many factors contribute; defining the role of each of these has proved to be problematic. Oxidation, and in particular that of low-density lipoproteins (LDL), has been linked to disease development (Steinberg *et al*. 1989) although the significance of this process has not been fully established, previous studies have variously implicated lipoxygenase, peroxynitrite, myeloperoxidase, oxygen radicals, and metal-ions in lesion oxidation (Stocker and Keaney, 2004). Zinc ions have been reported to modulate oxidant damage via the displacement of iron and copper from oxidation sensitive sites on erythrocyte membranes, LDL, or liposomes (Zago and Oteiza, 2001). Elevated zinc may stimulate the formation of oxidants and inhibit protective enzymes in some cells. Zinc supplementation, via dietary feeding, reduced lesion area despite insignificant changes in lesion zinc concentrations; these changes were ascribed to a displacement of iron by zinc. Zinc supplementation was also shown to reduce accumulation of cholesterol in the aorta, decrease the average aortic lesion cross-sectional area, and reduce a number of markers of cholesterol and lipid oxidation (Jenner *et al*., 2007).

2.7.7 ENYZMES

Zinc is distributed widely in plant and animal tissues and occurs in all living cells. It functions as a cofactor and is a constituent of many enzymes like lactate dehydrogenase, alcohol dehydrogenase, glutamic dehydrogenase, alkaline phosphatase, carbonic anhydrase, carboxypeptidase, superoxide dismutase, retinene reductase, DNA and RNA polymerase. Zn dependent enzymes are involved in macronutrient metabolism and cell replication (Arinola, 2008). Carbonic anhydrase is present in erythrocytes, kidney tubules, gastrointestinal mucosa and glandular epithelium.Zinc is associated with enzymes, both as part of the molecule and as an activator. In its structural role, Zn usually stabilizes the quaternary structure of the enzymes. Substantial quantities of firmly bound Zn stabilize the structures of RNA, DNA, and ribosomes. In 1939, Zn was demonstrated to be a constituent of the metalloenzyme carbonic anhydrase, which contains about 0.33% Zn. Today more than 200 Zn proteins are known, and several biological roles for Zn have been clarified, including those related to cell replicated and differenciation (Hambidge *et al.,* 1986).

In severe Zn deficiency, activities of plasma alkaline phosphatase; liver, retina and testicular alcohol dehydrogenase; connective tissue and fetal thymidine kinase; pancreatic carboxypeptidase A; and liver nuclear DNA-dependent RNA-polymerase may be depressed. Zinc, functioning in enzyme systems, is largely involved in nucleic acid metabolism, protein synthesis, and carbohydrate metabolism. In rapidly growing tissues, Zn deficiency greatly reduces synthesis of DNA, RNA, and protein, and hence, impairs cellular division, growth and repair. Zinc proteins are involved in the transcription and translation of genetic material, perhaps accounting for its essentiality to all forms of life. The levels and activities of Zn metalloenzymes and Zn-dependent enzymes in the tissues of Zn-deficient animals have been extensively studied. Histrochemical determination carried out disclosed reduced activities of several enzymes, accompanied by reduced Zn levels, in the testes, bones, oesophagus and kidneys of Zn-deficient rats, compared with those of restricted-fed controls. In the testes, lactic dehydrogenase (LDH). Malic dehydrogenase (MDH), Alcohol dehydrogenase (ADH) and NADU (reduced nicotinamide adenine dinucleotide) diaphorase; in the bones, LDH, MDH, ADH and alkaline phosphatase; in the oesophagus; MDH, ADH and NADH disphorase; and in the kidneys, MDH and alkaline phosphatase were decreased (Kirchgessner and Roth, 1981).

2.7.8 HORMOMES

Zinc has many biologically significant interactions with hormones. It plays a role in the production, storage, and secretion of individual hormones as well as in the effectiveness of receptor sites and end-organ responsiveness. Among the most notable effects of Zn deficiency on hormone production and secretion are those related to testosterone, insulin, and adrenal corticosteroids. Spermatogenesis and the development of the primary and secondary sex organs in the male and all phases of the reproductive process in the female can be adversely affected by Zn deficiency. The major abnormality in the male is testicular hypofunction affecting both spermatogenesis and the production of testosterone by the Leydig cells. Less is known concerning the effects of Zn deficiency on sex hormones in nonpregnant females (Underwood, 1981).

Impaired development and functioning of male reproductive organs is reported in rats, humans, calves, kids, and lambs. In lambs, testicular growth was greatly impaired, and spermatogenesis stopped within 20 to 24 weeks on a low-Zn diet (2.4mg/kg) (Underwood, 1981). Zinc is associated with insulin in the pancreas, and pancreatic concentrations are markedly reduced by dietary deficiency. Zinc-deficient rats have a reduced concentration of plasma insulin and a lower pancreatic release of immunoreactive insulin. With inadequate Zn, Flynn *et al.,* (1972) reported that ACTH did not stimulate corticosteroid synthesis, suggesting that ACTH is functionally dependent on Zn.

2.7.10 IMMUNE RESPONSES

Zinc plays important roles in the development and proper functioning of the immune system (Fraker *et al*., 2000; Ibs and Rink, 2003). Deficiencies in zinc can lead to decreased immune function, as demonstrated by reduced T cell function, lower antibody titers and other deficits. Zinc is essential to the integrity of the immune system. Deficiency cause rapid atrophy of the thymus, reduced circulating lymphocyte counts with the predominant influence on various T-cell functions. Virtually complete loss of lymphoid tissues including thymus, tonsils, and lymph nodes has been reported in patients suffering from acrodermatitis enteropathica, a hereditary disease characterized by impaired utilization of Zn. Diversity of effects on imunocompetence as a result of Zn deficiency is related to thymic hormone production and activity (Hambidge *et al.,* 1986). Zinc deprivation increases the susceptibility of endothelial cells to oxidant stress (Beattie

and Kwun, 2004). Zinc deficiency adversely affects the secretion and functions of cytokines, the basic messengers of the immune system. Zinc functions as an antioxidant and stabilizes membranes (Prasad, 2009).

2.7.11 BRAIN DEVELOPMENT, BEHAVIOUR AND LEARNING ABILITY

Changes in the concentrations of neurotransmitters in the brain have been reported in zinc deprivation, but may be secondary consequences of appetite reduction (Kwun *et al*., 2007). Zn deficiency during the critical period for brain growth permanently affects brain function. When this deficiency is imposed throughout the latter third of pregnancy, brain size is decreased, there is a reduced total brain cell number, and the cytoplasm nuclear ratio is increased, implying an impairment of cell division in the brain during the critical period of macro neuronal proliferation. In adult life male rats so treated display impaired shock avoidance, and female rats are significantly more aggressive at a high level of shock than adult female rats whose dams were Zn sufficient during pregnancy. When the Zn deficiency is imposed from birth to 21 days of age, brain size is diminished, brain DNA, RNA and protein concentrations are reduced, and impaired maze acquisition ability is evident in such animals when adults. Retarded brain maturation, as indicated by reduced total cerebullar lipid concentration, is evident in Zn-deficient suckling rats and a markedly lower rate of protein synthesis in the brain of Zn-deficient weanling rats has also been demonstrated (Sandstead and Evans1984).

2.7.12 PROTEIN AND NUCLEIC ACID METABOLISM

Zinc plays a vital role in protein synthesis and digestion (Murray *et al.*, 2000) and fetal growth is especially affected by lack of zinc and this may reflect the roles that zinc plays in DNA synthesis and nucleic acid and protein metabolism. Zinc regulates genes involved in signal transduction, responses to stress, reduction–oxidation reactions, growth and energy utilization, including a T-cell cytokine receptor (Cousins *et al*., 2003). Zn is involved primarily in nucleic acid and protein metabolism and hence in the fundamental process of cell replication. Mention has already been made of the role of Zn in collagen synthesis and in DNA, RNA and protein formation in the brain. Impaired DNA synthesis in the liver of Zn-deficient rats has been demonstrated in several studies (Kirchgebner and Roth 1981). The total protein and RNA contents of the testes of Zn-deficient rats are reduced and the testes of more severely Zn-deficient rats contains lower concentration of

Zn, RNA, DNA and protein and higher non-protein nitrogen (N) levels and ribonuclease activity than either the restricted-fed controls. Zn was found to be of prime importance in reverting the inhibition by EDTA of the expression of the genetic potential of these cells to synthesize the enzymes required for DNA synthesis and cell division. In other words the process of "gene activation" requires Zn.

Many years ago the proteolytic activity of the pancreases was found to be reduced in the Zn-deficient rat. Pancreatic carboxypeptidase was later shown to be a Zn metalloenzyme. Carboxypeptidase activity was then found to be appreciably reduced in Zn-deficeint rats and to return rapidly to normal with Zn therapy (Kirchgessner and Roth 1981). No evidence was obtained that this reduction had limited protein digestion or absorption, so that this particular defect is unlikely to be directly responsible for the growth arrest and impaired food utilization of Zn deficiency.

2.7.13 CARBOHYDRATE AND LIPID METABOLISM

Zinc is necessary for the proper functioning of many enzymatic systems, and the insulin system is probably the most important one as zinc is an integral constituent of insulin and an important constituent of plasma (Murray *et al.*, 2000). It also plays a significant role in various peptidases, esterases and dehydrogenases. It influences the immune system, DNA synthesis, cell proliferation, protein synthesis and the incorporation of iron into the haemoglobin (Arinola, 2008; Prasad and Strakova 2011). Zinc, as an agent has been shown to have many insulin-like effects at both the organismal and cellular level and has ability to stimulate the expression of gIucose-6-phosphate dehydrogenase (G6PDH) and any other enzymes in fatty acid or carbohydrate metabolism (Sauer, 1998).

2.8 ZINC REQUIREMENTS

Minimum Zn requirement vary with age and functional activities of the animal and the composition of the diet, particularly the amounts and proportions of the many factors, organic and inorganic, which affect Zn absorption and utilization. Zn requirements are also influenced by ambient temperatures, where these cause profuse sweating and consequent large losses of Zn in the sweat, and by parasitic infestation with its attendant blood, and hence Zn, losses. The criteria of adequacy employed can also be important. For example, the requirements following trauma and disease are higher than "normal".

Poultry

The minimum Zn requirement of chicks for growth and health are given as 35ppm when fed on soybean protein diets containing 1.6% Ca and 0.7% P (O'Dell 1997). Lowering the Ca to 1.1% slightly decreased this requirement but raising it to 2.15 had no effect. This estimate has S protein source total Zn requirements are lower. The minimum dietary level for Zn for growth in chicks can therefore be given as 35–40mg/kg for soybean protein-type diets and 25–30mg/kg on diets in which the protein comes mainly from animal sources. Furthermore, the chick is less vulnerable than the pig to excess Ca. There is ample evidence that the Zn intakes just given are also adequate to meet the requirements of egg production and hatchability. NRC (1994) recommends a level of 40-75mg/kg zinc in various poultry diets. Ajayi *et al*. (2011) indicated that 48.37 mg of Zn/kg is sufficient for early chick growth. Broilers were fed 80 mg of Zn/kg. Despite the fact that the estimate for Zn requirement varied depending on which response criteria was used, it is important to list only the best estimate of the requirement to meet all metabolic needs (Wedekind *et al.,* 2003). Thus, it is recommendation that the Zn requirement of the broilers from hatch to 21 d of age be 84 mg of Zn/kg, twice the recommended value of NRC (1994).

2.9 SOURCES OF ZINC

The sources include red meat, fish meals, liver, eggs, dairy products, vegetables and some sea foods (Soetan *et al.*2010).Underwood (1977) summarized information on the Zn content of animal feeds. Plant species vary widely in Zn concentration; legumes usually have higher concentrations than grasses. Industrial pollution can produce high concentration of Zn in waters. Galvanized pipe and other equipment contribute substantially to the animals Zn intake. The trend however is toward the use of plastics and other materials that supply little or no Zn. Poor drainage increases forage Zn. But increasing soil pH decreases Zn availability and uptake of Zn for plants. In most circumstances, Zn declines as plants mature. Climate may influence plant concentrations as prevalence of Zn deficiency in cool, wet seasons has been associated with decreased solubility of soil Zn. Certain impurities in commercial N: P: K fertilizers, such as Zn in superphosphate, will provide to plants significant quantities of the element. The cereal grains used as the basis of pig and poultry rations typically contain 20-30mg/kg Zn, with appreciably higher levels in most materials used as protein supplements. Typical values

for soybean, peanuts and linseed meals may be given as 50-70 mg/kg Zn. The Zn contents of fishmeal, whale meal, and meat meals are normally much higher than that of soybean meal levels of 90-100mg/kg Zn or more are common. The Zn concentration in plants usually falls with advancing maturity and leguminous plants invariably carry higher Zn levels than grasses grown and sampled under the same conditions. Heavy dressings with lime and to a lesser extent with super phosphate can greatly reduce pasture Zn levels (Underwood, 1981).

2.10 DEFICIENCY

Mineral deficiencies may be characterized as primary or secondary depending on the cause of their development. Primary mineral deficiencies are caused by diets that are naturally deficient in one or more minerals. These deficiencies are corrected by the addition of one or more minerals to the diet. Secondary mineral deficiencies are caused by the consumption of one or more mineral antagonist that prevents the absorption or metabolism of another mineral. Many other antagonistic relationships exist between different minerals. For examples, the antagonists for zinc include iron, copper, and calcium (Stevenson, 2009).

Zinc deficiency has been established experimental with a large variety of animals and under natural field conditions for pigs, cattle, sheep, goats, poultry, and humans. Reduced feed intake and growth rate are the first effects of a deficiency in the growing animal, followed by parakeratosis and hair loss. Depressed gonadal function and impaired immunity are characteristics of the deficiency in several species.

2.10.1 Effects of Deficiency

Poultry

Zinc deficiency occurs fairly widely in chickens, turkeys, and other poultry on practical diets. The age of the birds, the quantity and availability of Zn in their diet, dietary antagonists (i.e, phytates and Ca), and the availability of Zn in the environment (i.e, from galvanized Zn coated cages) all affect the possibility of Zn deficiency. Zinc deficiency is more likely in the young, particularly those from Zn-deficient hens. Generally, Zn deficiency in poultry is not severe. However, even moderate deficiency lowers the rate of growth, feed efficiency, and egg production. Zinc deficiency can cause retarded development and affect the color of the comb. Zinc deficient birds also lay their first eggs

much later than do similar birds on the same diet, but with supplemental Zinc. In young chicks the clinical signs of Zn deficiency include retarded growth; shortening and thickening of leg bones and enlargement of the hock joint; scaling of the skin, especially on the feet; very poor feathering; reduced feed efficiency; loss of appetite; and in severe cases, death (Scott *et al.*, 1982).

Failure of normal development of long bones with shortening and thickening of the tibia and tarso-metatarsus as well as enlargement of the hock has been observed in Zn-deficient embryos, micromelia, curvature of the spine, and shortened, fused thoracic and lumbar vertebrae are showed. Toes often were missing, and in extreme cases, the embryos had no lower skeleton or limbs in less severe cases of Zn deficiency the chicks sometimes hatched but were too weak to stand, eat, or drink. These chicks had an accelerated rate of respiration, and breathing was labored (O'Dell, 1997). In contrast to the other species, ducks that were severely deficient as judged by weight gain showed no distortion of bone proportions or of abnormal tarsal joints. In the Zn–deficient chick, abnormal posture, stiff gait, and reluctance to move suggest painful joints similar to those of arthritis in humans.

Swine

The main clinical sign of Zn deficiency in swine is the skin disorder parakeratosis. The typical skin lesion is characterized by excess keratinization of the epidermis with the formation of horny scale and fissures, accompanied by a brown exudates and alopecia. The lesion resembles that produced by the mange mite (*Sarcoptes scabies*), but is distinguished from mange by the rapid and dramatic disappearance of the lesions when the diet is supplemented with Zn. The keratinous crusts usually occur early in the pastern, fetlock, knee and hock regions of the legs. These are the first clinical evidence of the disease that is noted without catching and restraining the pig for close examination. For most cases the scale spreads until it affects a large area of the body. The scale is horny and dry on exposed surfaces and usually is easily removed. Occasionally, secondary infection in the cracks and fissures causes then to fill with a dark, sticky exudate. The skin lesions are accompanied by reduced appetite and growth rate. Additional signs are scouring, vomiting and death. In Zn-deficiency baby pigs, reduction of growth rate occurred before voluntary feed intake was affected. There are conflicting reports on the reproductive effects of Zn deficiency in swine. Wegger and Palludan (1978) indicated Zn deficiency resulted in some stillborn pigs while others died shortly after delivery,

apparently from excessive blood loss from the umbilicus, which failed to contract normally. Ligation of the cord often proved impossible owing to its unusual brittleness. He also studied Zn deficiency in boars. Testicular weight was reduced and histological examination revealed abnormal structure of testes.

Ruminants

Early effects of Zn deficiency include reduced feed intake, growth rate, and feed efficiency. Feed passes through the digestive tract more slowewwly in a Zn deficient animal and deficient animals grow more slowly than normal. Utilization of nutrients after digestion is impaired, including lower nitrogen and sulfur balances due to increased urinary excretion of these elements have indicated that excessive saliva is one of the earliest clinical signs in calves (Underwood and Suttle, 1999).

Parakeratosis of the skin is perhaps the most obvious clinical sign of severely Zn-deficient ruminants. In calves, the scrotum, head, and the area around the nostrils, neck, and legs most often are parakeratotic. In lactating cows, teats may show considerable parakeratosis. Parakeratotic changes have been observed in the papillae of the rumen and to the esophageal mucosa (Kirchgessner and Roth 1981). Zinc-deficient calves exhibited bowing of the hind legs and stiffness of the joints, corrected with Zn repletion, but the skeletal system is not affected adversely in cattle to the extent that it is in chicks and swine. Calves deficient in Zn grow more slowly and are lethargic: their wounds heal very slowly, if at all; and they are highly susceptible to nonspecific secondary infections. It is not entirely clear which of these lesions are direct effects of the deficiency, and which are secondary, because of the easily infected nature of the skin with testicular growth and development often retarded in Zn deficiency diets.

In sheep and goats the wool becomes loose, brittle, loses it crimp, often is lost, and may be eaten. With wool loss there is a development of thick, wrinkled, pink skin. The whole fleece may be shed, and no further wool growth occurs until additional dietary Zn is supplied; then regrowth is immediate. The horns and hooves of sheep and goats can be soft, deformed with swelling and lesions, and may lack normal surface striations. All phases of the reproductive process in females from estrus to parturition and lactation may be adversely affected. A number of factors, including soil, plant species, stage of maturity, yield, pasture management, and climate, may affect the likelihood of a Zn

deficiency for ruminants. Marginal Zn deficiency in grazing sheep and cattle, characterized by subnormal growth fertility, and low serum Zn values, but without other clinical signs, is more widespread than was earlier believed. The first effects of a mild Zn deficiency are decreased feed intake, growth, feed efficiency, and milk production, resistance to infection and stress, and reproductive efficiency (Underwood, 1981).

Rabbits.

Zinc deficiency signs include reduced feed intake, lowered hematocrict, weight loss, grazing of dark hair, alopecia, dermatitis, and reproductive failure (McClain *et al*., 1984). Unreceptiveness to the male and apparent failure of ovulation were factors in decreased fertility.

2.11 SUPPLEMENTATION

Zinc must be present in the diets of all animals and must be supplied almost continuously because animals have only small amounts of readily available stored body Zn. Reliance on tissue Zn to compensate for low intakes should not be expected to maintain maximal production rates. Dietary Zn source is important in determining whether an inorganic supplement is required. Animal protein sources, such as meat meal of tankage and fish meal are generally richer in Zn and of higher biological availability than plant-protein supplements. In general, all-plant diets should be supplemented with an inorganic Zn salt. Zinc intake may be increased when animals have access to forage or pasture that provides forage potentially rich in Zn and also allows Zn to be obtained through consumption of soil or plants with soil contamination. A number of factors other than dietary Zn concentrations will determine need for supplementation, principally dietary phytate and Ca concentrations.

Confinement systems for swine and poultry lowered Zn intakes from pastures and soils. Also, with confinement came the use of greater quantities of plant-protein sources, lower in available Zn, and greater use of Ca supplements, which increase Zn requirements. High Cu levels in diets, which are antagonistic to Zn, increase in Zn needs. Zinc supplementation of cattle diets may also become increasing important with greater use of high- concentrates feeds and protein supplements such as urea, which contain little Zn

(McDowell *et al.*, 2003). Human needs for supplemental Zn have increased for some of the same reasons as those of animals in confinement. The greater use of soybean products containing phytate and low- income groups consuming less zinc- rich animal products have made human nutritionists aware of the needs for Zn supplementation. Humans and animals that are fed milk substitutes that include components high in phytin (e.g soybean meal) have increased need for supplementation. Formerly most water pipe was galvanized, which resulted in water with a substantial quantity of Zn in relation to requirements, compared to newer water systems that now provide little Zn.

Supplemental Zn is easily and cheaply provided by fortifying complete diets, as mineral concentrates, mineralized salt, salt blocks, molasses blocks, and sometimes added in the water source. Premixes of Zn and other trace minerals may be added to feeds at the farm. Generally, such premixes contain from 0.5 to 10% Zn. A complete free-choice mineral supplement for cattle consuming approximately 50g/day should contain 1-2% Zn to provide a significant proportion of the Zn requirement. Many of the commercial trace-mineralized salt mixtures and free-choice mineral mixtures provide an insignificant amount of Zn relative to animal requirements. Zinc as the metal, sulfate, carbonate, oxide, and in several natural ores has been shown to be relatively available as supplements when provided in suitable physical form for mixing. The two predominant sources used by the animal feed industry are Zn oxide (72% Zn) and Zn sulfate (36% Zn). When a chick bioassay of total tibia Zn was regressed on supplemental Zn intake, bioavailability of ZnO was 44.1% compared with $ZnSO_4$ (set at 100.0%). Certain Zn ores, i.e., sphalerite (Zn sulphide) and franklinite (oxides of Zn, Fe, and Mg) are poorly available, as judged by their limited capacity to promote growth in Zn-deficient chicks. Zinc is normally found in combination with lead and other heavy metals, and must go through a series of purifications to remove the metals. A quality Zn oxide, for example, should contain no more than a maximum of 0.05% lead, 0.03% arsenic, and 0.001% cadmium. Studies with the dairy cows have shown an organic Zn compound, Zn methionine, as a promising nutritional feed additive. Dairy cows receiving Zn methionine produced more milk with lower somatic cell counts and had higher hoof quality than cows receiving Zn oxide and methionine separately (Wedekind and Baker, 1990).

2.12 ZINC TOXICITY

The presence of a mineral in animal tissues does not imply its essentiality. Minerals can be natural contaminants and, in this case, they follow a similar distribution as that found in the surrounding environment. Essential elements, however, exist in a normal and expected symmetry, and their deficiency results in impairment or loss of organic functions. The presence of essential elements in animal tissues follows cell function and, therefore, they have typical concentrations for each organ. As occurs with any other nutrients, minerals can cause toxicity if ingested in high levels or for long periods (Vieira, 2008). Zinc is relatively non-toxic to birds and mammals. Rats, pigs, poultry, sheep, cattle and man exhibit considerable tolerance depending greatly on the nature of the diet, particularly its content of calcium, copper, iron and cadmium, with which it interacts in the process of absorption and utilization. For this reason, studies of minimum toxic levels of dietary Zn are only meaningful when the status of the diet and the animal with respect to these interacting elements is known and defined.

Weanling pigs fed for several weeks diets containing 1000mg/kg Zn, either as the sulfate or carbonate, suffered no obvious ill effects. At higher Zn levels depressed growth and appetite, arthritis and internal hemorrhages were observed, and at 4000 and 8000mg/kg, mortality was high Raising the dietary Ca level from 0.7 to 1.1% had a protective effect against the toxic effects of 4000 mg/kg Zn (as ZnO) in a recent experiment with weanling pigs. Growth and appetite were better in the pigs fed the high-Ca diet, and the Zn levels in their blood and tissues were significantly lower than in those ingesting the diet lower in Ca. Broilers and layer hens exhibit a tolerance to Zn, similar to pigs at 1200-1400 mg/kg of the diet and a similar growth and appetite depression when the level is raised to 3000 mg/kg. The importance of diet composition on Zn toxicity is evident from the work of Berg and Martinson (1972). Little or no evidence of toxicity was observed when 2000mg/kg Zn as ZnO was added to corn-soybean, corn-fish meal, or sucrose-soybean diets fed for 2 weeks to baby chicks. The same amount of Zn added to a sucrose- fish meal diet reduced weight and bone ash and as little as 800mg/kg Zn was found to be toxic with this diet.

Sheep and cattle appear to be less tolerant of high Zn intakes than rats, pigs and poultry. Consumption by lambs of diets containing 1000mg/kg Zn as the oxide reduced weight

gains and decreased feed efficiency, and diets containing more than 1500mg/kg depressed feed consumption (Ott *et al.*, 1966). In a recent study, Campbell (1975) showed that diets containing 700mg/kg Zn offered to pregnant ewes resulted in high incidence of prenatal deaths in lambs. Steers and heifers have been shown to be unaffected by 500mg/kg Zn or less, but 900mg/kg caused reduced gains and lowered feed efficiency, and 17000mg/kg induced, in addition, depraved appetite characterized by excessive salt consumption and wood chewing. Subnormal liver Cu levels and a mild anemia occurs but liver Fe levels actually increase and defective development and mineralization of bone are not apparent, as reported for Zn-toxic rats. At the higher levels of Zn intakes changes in rumen metabolism evidenced by a reduction in the volatile fatty acid (VFA) concentration and acetic acid: propionic acid ration occurred in lambs, probably through a toxic effect of the Zn on the rumen micro organisms.

The relatively low toxicity of Zn among divalent cations, coupled with efficient homeostatic control mechanisms, make chronic Zn toxicity from dietary sources an unlikely hazard to man. Where Zn salts or compounds are given orally in large doses over prolonged period, as in the treatment of chronic leg ulcers or the prophylaxis of cardiovascular diseases, possibilities of toxic effects cannot be dismissed. Indeed doses of 150mg Zn/day, which are equivalent to about 200-300mg/kg of the total daily dry matter intakes of an adult, are enough to interfere with copper and iron metabolism, since Zn is a metabolic antagonist of both these metals

2.13 INTERACTIONS BETWEEN MINERALS

Trace minerals are usually supplied in commercial poultry diets as inorganic trace mineral salts (oxides or sulphates). However, it is known that the bioavailability of inorganic trace minerals can be low and variable due to an array of nutritional antagonisms, including dietary levels of phytic acid, fibre, Ca and P (Ravindran et al., 1995; Underwood and Suttle, 1999; Leeson, 2005). While little or no interference in the intestinal absorption of essential minerals is desirable, this is generally not the case. There are numerous antagonistic compounds in the diet that cause less than optimal absorption of many minerals. Also, certain minerals can interact with each other and mutually affect each other's metabolism (Figure 1) (Ashmead, 1993). .

Mineral absorption can suffer much interference, such as mutual antagonisms, which potentially reduce absorption and metabolism rates of some minerals. These interrelationships are traditionally expressed as shown in Figure 1, which simplifies how the interactions among minera ls can potentially affect each other. Insoluble precipitates can be formed through the competition with organic and inorganic ligands. Examples are phytic acid and phosphates. Both can reduce or completely inhibit mineral availability. Phytates reduce zinc uptake (Hempe and Cousins, 1989, (Ravindran *et al*., 1995; Underwood and Suttle, 1999; Leeson, 2005) and calcium impairs the absorption of copper and zinc (Lowel *et al*., 1994; Wedekind *et al*., 1994). Copper and molybdenum are strongly antagonic, whereas manganese and iron compete for similar absorption mechanisms. Manganese supplementation to solution containing iron depresses iron intake (Sandstrom, 1992). Iron, potassium and magnesium are potentially antagonistic to manganese. It was demonstrated that phytase supplementation improves trace mineral availability in monogastric animals; however, action of this enzyme can be limited by dietary calcium level (Angel *et al*., 2002). Many animals therefore require supplemental Zn in the diet for normal body function because of either low levels in the dietary ingredients or the presence of antagonistic factors which decrease the bioavailability of the element. Antagonism might be due to metal ion interactions such as with Fe or Cu. Source of fibre has also been reported to decrease the availability of Zn (Baker and Ammerman, 1995). Studies have confirmed the Zn x Cu x Fe interactions by demonstrating that the addition of Zn and Fe counteracts the effect of high Cu levels in the diet, apparently through reduction of absorption since accumulation of Cu in the liver is sharply reduced when Zn and Fe are added with the Cu to the diet. There is good evidence that this interaction in Zn x Cu x Fe in absorption from the gastrointestinal tract is primarily a competition for binding sites on transferrin and the metallothioneins (Davis, 1980).

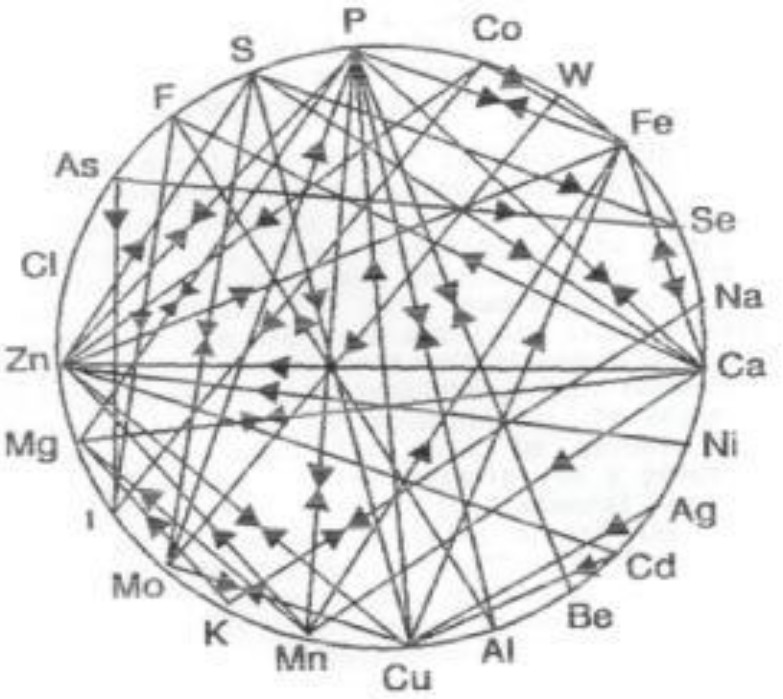


Figure 1: Potential interrelationships between minerals in intestinal lumen and metabolism

The arrows indicate antagonism between elements. For example Ca is antagonistic to Zn.

Source: Mineral interrelationships in animal metabolism (Ashmead, 1993).

Nutrients Synergistic to Zinc

Minerals that are synergistic with zinc include magnesium, manganese, iron and phosphorus. The following vitamins are considered synergistic to zinc: vitamin A, B1, B3, B5, B6, and vitamin E. It should be noted that some vitamins and minerals appear as synergistic as well as antagonistic. Within a physiological range a nutrient can act as a metabolic synergist to zinc, but in higher amounts it can antagonize the metabolic functions and/or absorption of zinc. As an example, even though the mineral copper is antagonistic to zinc, it can also be considered synergistic to zinc metabolically, since both are required for normal collagen synthesis integrity (David and Watts, 1988).

2.14 THE COCKEREL

The cockerel is a male chick, and a by-product of commerciall pullet production operation in hatcheries. It is also a meat- type chicken.

2.14.1 External Features

The basic external parts of a chicken include the comb, beak, wattles, ears, earlobes, eyes, eye rings, wings, tail, thighs, hocks, shanks, spurs, claws, and toes. Both male and female chickens have these basic parts. The differences between males and females include the size of the comb and wattles, the size of the spurs (in older birds), and the characteristics of the hackle and cape feathers. Hackle and cape feathers of males have pointed ends, whereas those of females have rounder ends. In addition, males have sickle feathers in their tails and hackle feathers on their backs, and females do not. The thigh **o**f a chicken is the upper part of the leg attached to the body of the bird. The thigh ends at the lower leg (drumstick). The thigh is connected to the shank (foot) at the hock joint, which is the equivalent of the ankle in humans. Chickens stand and walk on their toes**.** Most chickens have three toes projecting forward and one projecting back, sometimes referred to as the claw**.** (Small and backyard flocks, 2012). The external features are similar to other domestic fowls. It is a vertebrate with modification for flight. It is completely covered with feathers. The saddle, hackle and sickle feathers are narrower and longer than those in pullets. The shanks are covered with scales and are variable in colour. The comb and wattles are on and below the head respectively, the skin lacks sweat glands (Oluyemi and Roberts, 2000).

2.14.2 Internal Features

The skeleton is similar to that of mammals; its wings corresponding to the arms and hands while its legs and feet correspond in the domestic fowl to form the shank (Oluyemi and Robert, 2000). The breast muscles are well developed to motivate the wings and protect the vital organs of the chest and abdomen. They are of economic importance in commercial meat production. The rates of blood flow through the muscle determine their colour. The lungs are small and supplemented with air sacs, the lungs are active while the sacs are passive in respiratory exchange. In the digestive system, the crop is the storage pouch for food. The proventiculus is the glandular stomach where pepsin and hydrochloric acid are secreted by the glandular cells. The gizzard is the muscular stomach that functionally replaces the teeth. The small intestine is longer than the large intestine. The cloaca is the bulbous end of the alimentary tract where the urinary and reproductive tract also terminates (Oluyemi and Roberts, 2000). The reproductive system consists of two testes located on the dorsal area of the body cavity, just in front of the kidney.

2.14.3 MANAGEMENT OF COCKEREL

2.14.3.1 Housing

The environment to which the bird is exposed is determined partly by the systems of management and this includes the design of housing used (Oluyemi and Roberts, 2000). The houses should be constructed in such a way that the birds will not be exposed to adverse effect of temperature and humidity, thereby predisposing them to thermal stress. Management systems used in rearing cockerels include extensive, intensive and semi-intensive systems. The most common system used for large scale production is the intensive system. Attention should also be placed on spacing, so as to avoid over-crowding that comes with other vices like feather pecking, cannibalism etc.

2.14.3.2 Environmental Factors

Poultry thrives well when they are provided with conducive environment. The combined effect of high environmental temperatures and relative humidity prevalent in the tropics (than in the temperate countries) often results in thermal stress. Oluyemi and Robert (2000) reported the body temperature of the domestic fowl as 38.9^{o}C for day old chick and 41.9^{o}C for the adult with a small variation of $\pm 5^{o}$C and a range of 38.9-43.6^{o}C. The ability of birds to withstand high temperature depends on certain environmental factors

such as duration of exposure, rate of temperature change and the maximum temperature. The performance of birds is a result of the interaction between genotype and environment. The change in genotype is as a result of natural or artificial selection, gene drift and mutation. Therefore, performance is subject to explosure of birds to favourable environmental factors like temperature, nutrition, ventilation, pathogens, stress etc (Oluyemi and Roberts, 2000). To optimize production, focus should be on the environment. Animal breeders having realized this have made good genetic progress.

2.14.3.3 Diseases and Health Management

Disease is a deviation from the normal condition. Poultry diseases are major cause of financial losses. Diseases could be due to mechanical and thermal injuries, nutritional deficiencies, poison, hereditary and congenital. It is therefore important to carry out intensive management routine, as there are complex interactions between diseases and the environment. Some breeds are more resistant to particular diseases therefore selection of those breeds for improved resistance will reduce the heavy losses incurred in the poultry enterprise. Common diseases of poultry are coccidiosis, newcastle, fowl pox, gumboro etc. An accurate diagnosis of disease is very important for future planning than for immediate therapy (Reid *et al*., 1990). They further reported that the keys to controlling poultry diseases are prevention through vaccination, good nutrition and clean environment.

2.15 REPRODUCTIVE PHYSIOLOGY AND ANATOMY OF COCKERELS

Cockerels have two testes located in front of the kidney. The testes together weigh between 14 and 60gm (Hafez, 1993) depending on the breed and age of the bird. They are suspended from the dorsal body wall just posterior to the lung and ventral to the kidney. The seminiferous tubules of immature males are lined by a single celled layer of sertoli cells and stem spermatozonia, while mature males have irregularly shaped-tubules lined by a multi-layered germinal epithelium. The spermatogonia give rise to the primary spermatocytes, secondary spermatocytes and spermatids, which metamorphose into spermatozoa, Spermatogonia multiply about the 5^{th} week after hatching and primary spermatocytes appear about the 6^{th} week, and about the 10^{th} week, cells multiply rapidly, the secondary spermatocytes appear and the tubules increase in size. Spermatids first appear soon afterward and continued development in the tubules till the 20^{th} weeks,

hereafter; the testes appear capable of producing spermatozoa in large quantities. Spermatozoa are stored mainly in the duct deferens and there are no accessory reproductive organs such as seminal vesicle, prostate gland, cowper's gland and urethral glands. The male has no penis, but an erectile phallus, this is believed to make contact with the everted vagina during copulation.

2.15.1 GENERAL ANATOMY OF COCKERELS

2.15.2 SPERM PRODUCTION

Spermatogenesis is a continual process once puberty sets on. Inside the testicle, presperm cells, called spermatogonia, begin to mature near the outside wall of the seminiferous tubes. As the cell matures, it moves closer to the center of the tube where it will be released into the sperm passageway (lumen). During maturation, the sperm cell changes shape from a round shape to the elongated sperm head with a tail. There are many sperm cells in different stages of development. The time period required to change from a round cell on the outer wall of the tube to its release into the seminiferous tubule as a sperm with a tail takes approximately 34-36 days. The production of sperm cells in the testes is not synchronized within the tubules so that sperm cells can be produced for ejaculation on a daily basis. Therefore along the entire length of the seminiferous tubules there are segments that contain different stages of sperm cell development (De Kretser *et al.*, 1998).

The seminiferous tubules can be divided into three compartments: basal, adluminal and luminal compartment. The basal compartment consists of basal cell membrane, spermatogonia and sertoli cells. The adluminal compartment, in which differentiation of spermatocytes occurs, consists of primary and secondary spermatocytes, round spermatids and elongated spermatids. The luminal compartments consist of spermatozoa that were released from the sertoli cells. The basal and adluminal compartment are separated by intercellular structures between adjacent sertoli cells, which form a continous lock, so called the "blood-testis barrier" (Ritzen, 1983).

2.15.3 SPERMATOGENESIS

This is the sequence of cellular divisions and developmental changes that occur within the seminiferous tubules of the testes (De Kretser and Kerr, 1988; Eddy and O'Brien, 1994). The spermatogenetic process is comprised of two major processes.

(1) *Spermatocytogenesis* which contains two processes, mitotc process of stem cells (spermatogonia) to form spermatocytes and meiosis to reduce the number of chromosomes to form spermatids, and

(2) *Spermiogenesis*, which is the transformation of round to enlongated spermatids in regards to metamorphic changes.

Spermatocytogenesis and spermiogenesis are closely associated with sertoli cells, the nurse cells for spermatozoon inside the seminiferous tubules. The sertoli cells contribute to the blood-testis barrier and supply the nutrients needed for the spermatogenic process. The spermatogonia or stem cells comprise A0, A1, A2, A3 and A4 type of spermatogonia. The division of type A4 forms either A1 spermatogonia or intermediate spermatogonia and another division to form type B spermatogonia. The type B then divide mitotically to form pre-leptotene spermatocytes. When the spermatocytes enter meiotic division, the stage is then referred to the leptotene stage. At the end of the leptotene, the cells are called primary spermatocytes, which have duplicate number of chromosomes. The primary spermatocyte undergoes progressive nuclear changes of meiotic prophase, to form secondary spermatocyte (2N) and then the secondary spermatocyte undergoes meiosis II to reduce the chromosomal number to haploid (IN). The completion of meiosis requies the presence of heat-shock protein (De Kretser *et al*., 1998). Genetically modified animals with gene deletion failed to complete meiosis and increased apoptotic rate of spermatocytes. At the end of the meiotic process, spermatocytes form round spermatids, which will undergo a progressive and complex series of structural and developmental changes to form spermatozoa (De Kretser and Kerr, 1988; De Kretser *et al*., 1998).

Spermiogenesis is a series of progressive changes in structure and morphology of spermatids. The changes include:

1. Formation of spermatozoa flagella and development of a core of microtubules,
2. Nuclear chromatin condensation and movement of the nucleus to the periphery of the cell,
3. Formation of the acrosomal cap to form the acrosome, and
4. Formation of residual body by shedding a large part of cytoplasm (De Kretser and Kerr, 1988; De Kretser *et al*., 1998).

2.15.4 SPERMATOGONIA

The Spermatogonia lie at the base of the seminiferous epithelium. The spermatogonia act as stem cells, the type A spermatogonia are proliferative and transform into differentiating and simultaneously renew their population by mitotic replication. The replications of the spermatogonia are dependent on the expression of c-kit protein synthesized by sertoli cells (De Krestser *et al*., 1998). The type A spermatogonia can be classified as A1, A2, A3, and A4 and In (intermediate), according to the differentiation process. Type B spermatogonia are the final differentiated stage before the meiotic phase occurs in the spermatocytogenesis process (Russell *et al*., 1990). The differences among these types of spermatogonia are based on structure and morphology, using either light or electron microscopy.

2.15.5. PROCESS OF SPERMATOZOA MATURATION

Mature spermatozoa are released from the sertoli cell into the luminal compartment in the seminiferous tubules. The spermatozoa are transported to epididymal duct through the vasa efferentia by the flow of testicular fluid secreted from sertoli cells and intrinsic smooth muscle contractions of the tubules and testicular capsule (Moore, 1995). The epididymis is a single highly convoluted duct, which can be divided into three segments, anatomically, of head (caput), body (corpus), and tail (cauda). A number of functions of the epididymis have been reported, which include absorption of large volumes of fluid secreted into the rete testis by the seminiferous tubules, secretion of a variety of ions, small organic molecules, and glycoproteins into its lumen, biosynthesis and metabolism of steroids, and spermiophagy, and the phagocytosis of degenerate or dead spermatozoa. The epididymis also forms tight junctions throughout the duct, which is believed to be “blood-epididymis barrier” (Robaire and Hermo, 1988). The main function of the epididymis is the maturation of spermatozoon released from seminiferous tubule. The

process of epididymal maturation involves changes in several morpho-functional aspect of the spermatozoon (Briz *et al.*, 1995). The epididymal maturation is a gradual process, not an abrupt event. The basic maturation process involves the following.

1. Progressive loss of water by absorption of large volumes of fluid secreted into the rete testis by the seminiferous tubules,
2. Changes in the metabolic patterns and the structural condition of the tail,
3. Modifications in patterns of movement,
4. Detachment of the cytoplasmic droplet,
5. Modifications of the plasma membrane and
6. Changes in the nuclear chromatin and modification of the acrosome (Briz *et al*., 1995).

A number of spermatozoa structures become stabilized by disulfide bond formation, including flagellar structures that contribute to gain motility during epididymal transit (Katz, 1983). The capacity to fertilise is increased as the spermatozoa move down the epididymal duct. Incubation of caput spermatozoa with either cauda fluid or seminal plasma did not increase fertilizing capacity of spermatozoa in pig (Holtz and Smidt, 1976). The spermatozoa require transit through the entire epididymal duct to achieve fertilizing capacity.

However, although they are capable of movement, they are still too concentrated to permit motion. As the sperm move into the tail of the epididymis, additional proteins are added which are important for sperm fertility. Sperm are stored immotile in the tail region in concentrated form. The entire duration of the trip through the epididymis requires between 12-14 days. The sperm will acquire full motility and fertilizing capability when diluted with seminal plasma in the ejaculate. Therefore, when considering the total time needed for a sperm cell to begin development until it appears in the ejaculate, requires approximately 45 days.

2.16 Nutrient Requirement of Chickens

Diet is an important component of the environment under all climatic conditions. The potential of the birds cannot be attained if the environmental and not ably the nutrition are sub-standard (Oluyemi and Roberts, 2000). The nutrient requirement of an animal is the

amount of nutrient that must be supplied in the rations to meet the need of that animal for health and reproductive purposes (Ranjkan, 1981). The term 'requirement' is the quantity of a nutrient or of energy that should be supplied in the diet to meet the net requirement of the bird. The net requirement of a normal healthy chicken is the quantity of a nutrient or of energy that it should absorb; given a completely adequate diet in an environment compatible with good health, in order to meet its need. The nutrients required for metabolic and productive activities differ between classes of poultry and vary with age, sex, environment for the same species of bird (Njike 1979, Fetuga, 1984,), stage of production, strain and temperature (Oluyemi and Roberts, 2000).

The nutrient requirement of chickens are defined more precisely than for other domestic animals. This is so because of the nature of birds, short generation interval, specific environmental conditions and their prolificacy which has allowed for the collection of more information than with other species (Card and Neshein 1972). All chickens qualitatively have the same nutritional requirements. They need about 40 nutrients, these consists of 13 important amino-acids, 13 vitamins, 13 essential inorganic elements, linoleic acid and sufficient non-specific nitrogen to allow for production of the non-essential amino acids and enough metabolisable energy (ME) to meet the energy needs for maintenance and production (Scott, 1974).

2.16.1 Protein

This is a body building organic compound. Growth rate depends on protein intake. To meet the protein requirement of an animal, the essential amino acids must be supplied in proper quantity and quality and total level of nitrogen in the diet must be high enough to permit the synthesis of the non-essential amino acids (Patrick and Schaible, 1980).

Rhodimet (1993) reported that cockerels require a crude protein requirement of 18% and 15% at 0 to 6 weeks, and 7 to 20 weeks respectively. This is in agreement with the findings of Babatunde and Fetuga (1976). Auckland and Fulton (1973) observed that lowering the level of protein in the diet containing the same amount of metabolisable energy fed ad-libitum reduces the protein intake and growth rate, which eventually delays sexual maturity. Leeson (1990) stressed that most researches aimed at ensuring adequate

nutrient intake during adverse condition centers around manipulation of protein and amino acid specification.

Keshavarz (1984) recalled that dietary protein can be decreased during the growing and laying period with no ill effects on production if birds are fed with ration containing adequate and balanced amino-acid. Lesson (1990) confirmed that early growth rate (0 to 8 weeks) is likely more sensitive to amino acids intake than to energy intake. Oluyemi and Roberts (2000) recommended a crude protein of 23, 16 and 18% to 0 to 8 weeks, 9 to 20 weeks and 20 weeks and above respectively for cockerels. They also recommended methionine + cystine (%) of 0.8, 0.7 and 0.6, Lysine (%) of 1.0, 1.0 and 0.75 respectively. Olomu (1995) recommended 20, 16 and 14% at 0 to 8 weeks, 8 to 16 weeks and 16 to 20 weeks respectively for cockerels.

2.16.2 Energy

Birds eat to meet their energy requirement (Say, 1987 and Kreager, 1988) and energy is required for optimum performance. The performance of birds does not improve when the energy in the diet exceeds the optimum level. Therefore, a balanced ration must contain good quality and adequate quantities of protein, carbohydrate, fats, minerals and vitamins. Since the chicken eats primarily to satisfy its energy requirements, it becomes evident that the energy content of the feed determines within limits how much feed the chicken will consume. It further determines how much protein, minerals and vitamins should be in the feed. Hence, energy requirement is the only single nutrient requirement that sets the base for the quantities of all other nutrients in the diet (Oluyemi and Roberts, 1979).

Scot *et al.* (1974) observed that the rate of feed consumed was determined by the energy level of the ration. Rhodimet (1993) recommended 2,000 and 2,750 kcal/ME/kg for cockerels of 0 to 6 weeks and 7 to 20 weeks respectively. Reid and Weber (1975) observed that feed intake was significantly decreased with increasing dietary energy. Oluyemi and Roberts (2000) recommended energy intake of 3,000, 3,000 and 2,800 to 2900 kcal ME/kg for cockerels at 0 to 8 weeks, 9 to 20 weeks and 20 weeks and above respectively, they observed that at high temperature, energy consumption is decreased, and concluded that the dietary energy must be adjusted to arrive at an optimum dietary

energy to protein ratio. Olomu (1995) recommended energy of 2650kcal /ME/kg, for cockerels at 0 to 8 weeks, 8 to 16 weeks and 16 to 20 weeks respectively

Oluyemi and Roberts (1979) indicated that the Nigerian local fowl may have requirements different from adapted exotic strains under Nigerian conditions although he did not find any significant genetic diet interaction. At very low energy concentration, the brids may not meet its energy requirement and at high energy concentration may consume, more than is required for maximum growth rate and excessive energy may be deposited as fat. Olomu (1979) also observed lower performance when energy levels similar to those in temperate countries were used for birds in the topics. This opinon has a strong basis on the widely accepted view that high ambient temperatures and high energy levels depressed feed intake. More birds die on high energy diet than those on low energy. It was suggested that energy in feed should be lower for birds in the tropics than those for the temperate regions.

Obioha *et al.* (1982) observed that high ambient temperature and high energy levels depress feed intake and thus lowering production. This priority accorded energy requirements soon led to the detriment of meeting the requirement for crude protein. It becomes necessary that energy and crude protein should be related in dietary composition. This leads to the concept of calorie: protein ratio. Combs *et al.* (1982) defined the calorie protein ratio of a diet as the metabolisable energy (ME) divided by the percentage crude protein of the diet. The higher the protein contents at each energy level, the higher the growth rate. Calorie: protein ratio for a diet is important for its influence on growth feed conversion and carcass composition (Praps and Bird, 1983).

2.16.3 Vitamins and Minerals

For optimum performance the mineral and vitamin requirements have to be met. Oluyemi and Roberts (2000) recommended 1,500 i.u of vitamin A, 200 i.u. of vitamin D for starters and growers respectively of Calcium 1.0 and 0.8%, Phosphorus of 0.7 and 0.4% and Sodium of 0.15 and 0.25% for starters and growers respectively. The vitamins and minerals requirements are usually met by the addition of vitamin and mineral premix.

2.16.4 Fiber

Poultry unlike ruminants cannot tolerate high level of crude fiber (CF). Oluyemi and Roberts (2000) recommended 1.25, 2.5 and 5.0% CF at 0 to 8 weeks, 9 to 20 weeks and adult birds respectively. Fiber increases the bulkiness of a diet when in high concentration, limits feed intake and places a limitation on intake of digestive nutrients. Longe (1984) reported that addition of fibrous farm waste by products caused a reduction in metabolizable energy of basal ration from 22.89 to 9.31-11.21 MJ/kg. Acton *et al.* (1982) observed that decreased dietary fibre resulted in decreased apparent digestibility. This was attributed to the laxative influence of fiber, resulting in increase faecal weight and faecal nitrogen. Trait and Wright (1990) observed that reduced bioavailability of mineral might be due to the impaired intestinal absorption brought about by fiber-based diet.

2.16.5 Water

Water is essential in poultry nutrition. Akinwunmi *et al*. (1979) reported that chicken will die within 24 hours if not given water but may survive a little longer without feed. Hocking *et al.* (1997) observed that water intake is closely linked to feed intake. Water makes up about 85% of the body weight of chicken (Reid *et al.*, 1990), Smith (1991) observed that the bodies of adult birds contain 60% water. Water consumption is important for the maintenance and growth function in chicken. (Hunton 1986) reported that water requirement would be greatly inflated during hot weather. Smith (1991) found out that increased protein level increased water consumption. Inclusion of Sodium chloride in the diet increases water intake. Say (1987) reported that any shortage of water is accompanied by a reduction in feed consumption.

At present, there are no fixed quantitative requirements for water. There are too many factors, which influence the birds' need for water to permit the establishment of firm requirements. These include diet and physical form of the diet, inhibitors in feed ingredients, carbohydrate sources, age, breed, rate of production, environmental temperature and contaminants in feed and water systems. A rule of thumb proposed by Scott (1974) is that birds consume 1.5-2 times as much water as they do feed.

2.17 THE BLOOD:

2.17.1 FUNCTIONS AND SIGNIFICANCE IN NUTRITIONAL STUDIES

The blood is a fluid tissue that circulates through vascular channels to carry nutrients to cells and waste products to excretory organs. The total volume of circulating blood is kept remarkably constant, and is expressed relative to body weight (% or ml/kg). In general, blood volume of large domestic animals is approximately 8 to 11% and that of common laboratory animals, from mice to monkeys is approximately 6 to 7% of body weight (Deldar, 1998). Blood is a tissue which consists of a variety of cells suspended in a fluid medium called plasma (Wheater *et al.*, 1987). The characteristic red colour is impacted by hemoglobin.

According to Bentic-Smith (1974) and Kronfeld and Medway (1975), blood is the transport medium for the movement of nutrients to the cells and excretion of metabolic wastes through the kidneys, guts, lungs, liver and skin thus an important means of maintaining the homeostasis of the body. Breazile *et al.* 1971, Green, 1972; Sturkie, 1977 reported that blood helps to regulate body temperature maintaining a constant concentration of water and electrolytes in the cells regulating the body's hydrogen ion concentration and providing defense against microorganisms invasion. It is also responsible for the transportation of hormone from the sites of production organs for necessary action through the body and the transmission of the chemical integrators of the body. Aletor and Egberongbe (1992) reported that the various functions of the blood are made possible by the individual and/or collective actions of its constituents.

2.17.2 Blood Composition

The blood, which has been shown to be a guide to nutritional status in feeds (Wilson and Medd, 1978), contains a myriad of metabolites and other constituents, which provide a valuable medium for clinical investigation and assessment of nutritional status of human beings and animals. Generally blood consists of cellular components (blood cells) and a protein rich fluid component (plasma). The cellular components contain erythrocytes (red blood cells), leukocytes (white blood cells) and thrombocytes (platelets) and. The non-cellular/plasma component contains 91 to 92% water 8 to 9% solutes (e.g. proteins, lipids

(fats), carbohydrates, non-protein nitrogenous materials and electrolytes (Deldar, 1998) and other substances such as hormones, antibiotics and various enzymes (Nagabhushanam, 2002). The concentrations of these constituents are influenced by diet, metabolic demands and the levels of hormones and vitamins (Church *et al.,* 1984).

2.17.2.1 Importance of blood cell evaluation

Blood is an important index of physiological, pathological and nutritional status of the animal. Hence, blood constituents are widely used in nutritional evaluation and survey of animals (Olorede *et al.,* 1995). Therefore, for an organism to function properly it must be able to keep its blood composition relatively constant under normal condition and in stress situation through continuous anabolism, catabolism and renewal in all body tissues in the blood (Harting *et al.,* 1981). As blood is an important index of physical and pathological changes in the organism, such changes can be evaluated only if the normal values are known. There are some variations in the normal values within specie of animals, but much of these variations can be explained by the lack of standard method and by differences in age, sex, number and strains (Laird *et al*., 1970; Schalm, 1975). It should however be emphasized that unless the experimental animals are kept under carefully controlled conditions, a range of haematological values may vary because of spontaneous or latent disease (Sutherland *et al.,* 1958), genetic factors (Laird *et al.,* 1970), diet (Sturkie 1977) and other environmental conditions.

In Nutritional assessment (diagnosis), blood metabolites provide a clue for chemical studies of animal nutritional status. This is because it has been established that feed components affect blood constituents (Harper *et al.,* 1979). The relevant parameters of interest normally measured are the haematological, sero-biochemical, sero-enzymological, and sero-eletrolytical parameters.

The commonly used haematological includes include Red blood cell counts (RBC), Packed cell volume (PCV), Haemoglobin concentration (Hb), White blood cell counts (WBC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) are values derived from the erythrocyte counts Hb and PCV (Schalm 1971) while the biochemical values usually measured include Serum total protein, Albumin, Globulin, Albumin/globulin ratio,

Cholesterol, Triglyceride, Urea, Creatinine, Glucose, Bilirubin, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) which are muscle, liver and bone enzymes respectively. Biochemical indices also reflect circulation rhythm, gender, age, hormonal changes and organ disease that influence absorption, transport, metabolism and excretion of nutrients. Hence serum biochemical values are interpreted with attention to these factors.

2.17.3 Formed Elements of blood

The formed elements of the blood include red blood cells, white blood cells and the blood platelets. Because the red blood cells and platelets both lack nuclei, they are not typical cells

2.17.3.1 Erythrocytes (Red Blood Cells)

Mattew and Robert (1990) described an erythrocyte as a nuclear flexible biconcave disc that transport oxygen to body tissues and receives the waste products of metabolism for transport to the organs for excretion (Schalm *et al.*, 1975). The red blood cells of birds are flat with a large nucleus. Health and Olusanya (1985) reported that the normal value of RBC in domestic fowl is 30 x 10^6/mm^3. Orji *et al.*, (1987) reported that the red blood cells are higher in males than in females. Erythrocytes and other cellular components of the blood of chickens are nucleated like the blood of reptiles, amphibians and fishes. The cells are elliptical in outline and biconvex in profile with a mean diameter of 12 x 7.5µ. The colour of the individual cells as in lower vertebrates is yellowish green. In the blood of chickens the type of erythrocyte known as the reticulocyte is found. Frequency such cells are present as a fairly high percentage (7-8% of the red cells). The RBC counts for chickens varies from 1.5 to 4.5 million/mm^3 which is in good agreement with previously reported values.

The normal physiological functions of erythrocytes are gas exchange, participation in the buffer system of the blood and a role in the clotting mechanism (Hodges, 1977). The number of red blood cells varies from species to species because of physiological variations or certain pathological conditions. The number of erythrocytes in chickens is influenced by physiological conditions, sex, age, nutrition, laying performance, moulting, and physical exercise. Adult males have higher erythrocyte counts and slightly lower

platelets counts than young chickens. In freshly hatched chicks, the erythrocyte count is lower and after 7 days the values of adult chicken are reached (Mitruka and Rawnsley, 1981). Oyewale (1987) reported that Nigerian domestic chickens have lower RBC counts than those of the temperate breeds even though their PCV values were comparable. Also Awotwi (1990) reporting on the effect of climate on haematological values stated that RBC count was lower in the local domestic chicken in the tropics when compared with temperate chickens.

The increase in the number of RBCs is called polycythemia. Temporary polycythemia (erythrocytosis) may be caused by dehydration (from fever, loss of fluid etc) or by anoxemia (high altitudes, pulmonary and cardiac disease, met-haemoglobinemia). Anaemia results if either the number of functional red cells or the quantity of haemoglobin is decreased much below normal. Anaemia may be due to deficient blood formation because of poor nutrition, including dietary deficiency of iron, copper, vitamins, amino acids, loss of blood due to haemorrhage from wounds or because of parasites such as stomach worms or lice. It is also caused by deficient secretion of intrinsic factors from the stomach; this factor makes vitamin B_{12} absorption possible. Anaemia also occurs when blood cells are being haemolysed faster than new ones to replace them, or if the red blood cells fail to mature normally. Anaemia has a considerable effect on the cardiovascular system. Obviously the oxygen carrying capacity of the blood is decreased. In addition, a reduced concentration of RBCs means the viscosity of the blood is reduced, and therefore it flows faster. Therefore, anaemia in birds can result from haemorrhage, increased erythrocytes destruction or decrease erythrocyte production.

2.17.3.2 Haemoglobin Concentration (Hb)

The presence of haemoglobin within the erythrocyte is responsible for its ability to transport oxygen and for the red colour of the erythrocytes. Chemically haemoglobin is an iron containing conjugated protein (Heme + Globin, globular protein consisting of four amino acid chains) which has the physiological function of transporting oxygen and carbon dioxide. Each molecule of haemoglobin consists of one molecule of globin linked to four heme molecules and each is able to reversibly bind four molecules of oxygen to form oxyhaemoglobin. Because of the presence of haemoglobin blood can carry about 60

times as much oxygen as a similar quantity of water under the same conditions (Coles, 1986). The measure of Hb is the amount of oxygen carrying protein contained within the red blood cells (Swindle *et al.,* 2003). Haemoglobin concentration is measured in grams per 100ml of blood. In normal chickens, the haemoglobin concentration varies from 7.40 and 13.1g/dl. Males have a slightly higher haemoglobin concentration ranging from 7.50 to 13.1 than females 7.40 to 12.2 (Mitruka and Rawnsley, 1981), while Maxwell *et al.* (1990) recorded 10.6g/dl.

2.17.3.3 Packed cell volume (Haematocrit)

Haematocrit (PCV) measures the proportion of red blood cells to plasma in the peripheral blood but not in the entire circulation. It is a term that means the percentage (by volume) of whole blood that is constituted by red blood cells. Upon centrifugation, blood is separated into three distinct parts. The mass of erythrocytes at the bottom which is referred to as packed cell volume (PCV) and a white or gray layer of leukocytes and thrombocytes (platelets) immediately above the red cell mass which is most commonly referred to as the buffy coat, representing about 10,000 WBC/mm^3 of blood. In leucopenia and thrombocytopenia, the layer will be thin, whereas in leukocytosis or thrombocytosis it will be thicker.

The haematocrit is used for calculation of blood constant and as a check on the blood cell count because it measures the concentration of red blood cells. Although it is not an accurate measure of blood volume, the degree of haemoconcentration in shock associated with surgery, trauma and burns can be judged by the haematocrits. In conditions associated with hydremia such as pregnancy and excessive administration of fluids, the haematocrit values will be below normal. The PCV is decreased in anaemia and it is increased in primary and secondary polycythemia. This also shows variation with age and breeds. lncrease in PCV value indicates an increasing blood viscosity, which results in disturbed blood flow. Lower concentration indicates low blood viscosity and results in increased blood flow and lower blood pressure (Frandson, 1981); Jain (1986) gave the normal PCV range for domestic fowl to be between 22 to 35%. It is higher in the cock than in the hen. The value of PCV ranged between 23-55ml% (Mitruka and Rawnsley, 1981; Maxwell *et al.*, 1990) for normal male and female chickens.

It has been shown that the hot humid climate of the tropics depresses erythrocyte production in chickens (Oyewale, 1987). Awotwi (1990) reported 38.52%, 9.22 g/dl, 2.54 x 10^6/mm^3 and 25.44 x 10^3/mm^3 for the mean PCV, Haemoglobin (Hb), RBC count and Total white blood cell counts respectively for the adult local domestic chickens. Haemoconcentratioen is the opposite of anemia, which means that the ratio of red cells to fluid is above normal. Thus is indicated by an excessively high red cell count or high haemotocrit value. The total number of red cells in the body may be increased (a condition called polycythermia) or there may be a decrease of fluid. Either a lowered intake of water or excess loss of water can cause haemoconcentration which then is a result of dehydration. Vomiting and diarrhea as well as diseases causing high temperatures if continued over a long period, result in dehydration (Schalm *et al.,* 1975). To correct dehydration it may be necessary to supply water to the animal in the form of physiological saline or glucose solution parenterally. This means that it is given by some route other than by mouth, since an animal that is vomiting may be stimulated to vomit even more upon drinking water.

2.17.4 White Blood Cells (Leukocyte counts)

The blood stream serves as a medium of transport for the leukocytes from its origin to that part of the body in which it is to fulfill its ultimate function. Leucocytes are amoeboid cells with prominent nucleus. They are devoid of haemoglobin and exhibit amoeboid movements by means of which they are able to pass through the walls of blood vessels (Nagabhushanam 2002). An evaluation and interpretation of a total and differential leukocyte count without completing a physical examination of the animal would be an analogous situation. It is difficult if not impossible, to accurately interpret results in absence of all readily available information (Coles, 1986).

According to the author, the total and differential leukocyte count if properly interpreted is of value in confirming or eliminating a tentative diagnosis, as an aid in making a more accurate prognosis and results may also serve as a guide to therapy. The results of such an examination are reflective of susceptibility of the host, virulence of the infecting agent, nature and severity of the disease process, systematic response of the individual and duration of the disease process (Coles, 1986). Differential counts indicate the percentage of each type of white cell in the blood sample. The average leukocyte counts in birds are

about 20,000/mm^3 (20.0 x 10^3/mm^3), which is higher compared to counts of humans and other mammalian blood (Mitruka and Rawnsley 1981). Epelle (1982) reported a range from 24.4 to 32.1 x 10^3/mm^3. Jain (1986) reported the range of total leucocytes count for domestic fowl as 12.0 to 30.0 x 10^3/mm^3.

2.17.4.1 Functions of Leukocytes

According to Coles (1986), accurate interpretation of leukocyte counts is dependent upon an understanding of the basic concepts with respect to functions of the various leukocytes as well as some comprehension of the kinetics associated with the various types of leukocytes. On the basis of the presence or absence of refractile granules, the leukocytes are divided into two types; granular types (granulocytes) and non-granular type (agranulocytes) (Nagabhushanam, 2002). Leukocytes differ considerably from erythrocytes in that they are nucleated and are capable of independent movement. The life span of the white blood cells (WBCs) varies considerably from only a few hours for granulocytes to potentially months for monocytes and years for lymphocytes. In the blood stream itself, most of the white blood cells are non-functional and are only being transported to tissues when and where they are needed (Coles, 1986).

2.17.4.2 Granulocytes

They are characterized by the presence of specific types of granules in their cytoplasm (Nagabhushanam, 2002) that stain with common bloodstains, such as Wright's strain. These strains contain an acid dye eosin, which is red, and a basic dye methylene blue, which is bluish. Under normal conditions, granulocytopoiesis is a cell renewal system in which cell production equals cell death. This normally occurs in an orderly fashion from the blast cell to the mature granulocyte, which makes morphological identification of cell compartments relatively easy. Granulocytes are produced in the bone marrow; subsequently released into the peripheral blood and from there migrate into the tissue where they have their principal function (Coles, 1986).

When granulocytes enter the peripheral circulation, they immediately equilibrate between the circulating granulocyte pools (CGP) and marginal granulocyte pool (MGP). Combine pools of these is referred to as the total blood granulocyte pool (TBGP). According to the

nature of the granulation, granulocytes have been subdivided into three subgroups: neutrophilic, eosinophilic and basophilic granulocytes (Nagabhusbanam, 2002).

(i) Neutrophils

Neutrophils contain granules that stain indifferently and are not notably red or blue. They constitute the first line of defense against microbial infection by migrating to any area invaded by bacteria passing through the vessel walls and engulfing the bacteria to destroy them. In tissues, Coles (1986) reported that, the principal function of the neutrophilic granulocyte is phagocytosis of small particles. Neutrophils play an important role in bacterial phagocytosis and in modulating the inflammatory process and subsequent tissue damage (Deldar, 1998).

The neutrophils constitute the greatest number of all the WBC. They reside to a great extent along the inner margin of the capillaries and small vessels - a phenomenon called margination. When tissue injury occurs, the neutrophils are mobilized from their marginal sites to the injury area, and they squeeze through the capillary walls between the cells (diapedesis) where upon they migrate by amoeboid movement into the tissue to phagocytise the foreign particles (Schalm *et al.*, 1975). Neutrophils also play a role in coagulation, fibrinolysis lymphocyte activation and cytotoxicity (Delder, 1998). The neutrophilic polymorphonuclear leukocytes vary in size from 9-12µ and are the most numerous of white blood cells (Nagabhushanam, 2002)

(ii) Eosinophils

Eosinophils have as their primary function detoxification (Coles, 1986). According to the author, they are mostly commonly encountered in the epithelial lining of the intestinal and respiratory tracts where they are thought to function also as detoxifiers. Eosinophils are mobilized at the site of antigen-antibody reactions and this mobilization is accompanied by an increase in the number of eosinophils in the blood Stream. Delder (1998) reported that eosinophils play a major role in controlling parasitic infestation and regulating allergic and inflammatory processes.

(iii) Basophils

Basophils like eosionophils; have limited phagocytic and bacteriacidal capacities: Dvorak and Dvorak (1979) and Jones (1993) reported that basophils play a major role in allergic and inflammatory reactions, lipid metabolism and blood coagulation.

2.17.4.3 AGRANULOCYTES

(i) Lymphocytes

Lymphocytes are variable in size and appearance and have a relatively large nucleus surrounded by a small amount of cytoplasm. One of the major functions of lymphocytes is the response to antigens (foreign substances) by forming antibodies that circulate in the blood or in the development of cellular immunity. The sequence of development of lymphocytes is classically the same as other leukocytes beginning with a stem cell, progressing from a lymphoblast through the prolymphocyte stage to the final development of the mature cell which may be designated as either a large or small lymphocyte (Coles, 1986). In the adult animal, peripheral lymphoid organs such as spleen, lymph node and lymphoid related to the intestinal tract appear to be responsible for a majority of lymphocytes. However, according to Coles (1986), lymphopoiesis continues to occur in the bone narrow independently of antigen stimulation. The blood

distributes majority of the cells produced in the bone narrow to peripheral lymphoid organs. Lymphocytes in the animal body are constantly in a state of circulation and recirculation because of this constant recirculation and the fact that there are populations of lymphocytes in varying life span; Coles (1986) stated that it is not possible to precisely determine the total number of lymphocytes in an animal body at any given time. The factors regulating blood lymphocytes level are also largely unknown although antigen stimulation may result in an outpouring of reactive lymphocytes from lymphoid tissues. This is a transient phenomenon and not frequently observed as a clinical-entity. The principal function of the lymphocyte is in relationship to its immunological activity. It circulates throughout the body and plays an important role in immunologic defense for the host. It is also involved in the regulation of haematopoiesis, takes place in cellular immunity and is involved in cell-mediated cytoxicity and antibody-dependent cellular toxicity (Deldar, 1998).

(ii) Monocytes

Monocytes, the largest white blood cells like neutrophils, are phagocytic, that is, they have the ability to engulf foreign matter such as bacteria. However, while the neutrophils act mainly in overcoming acute infections the monocytes are called into action by less acute infections such as tuberculosis. When monocytes from the blood enter tissues, they develop into larger phagocytes called macrophages.

Monocytes arise predominantly, if not exclusively, in the bone marrow from a system of progenerators comparable in behaviour with tissue of other blood cells (Volkman, 1970). Monocytes, unlike granulocytes, retain their nucleoli as they mature, thus suggesting that mature monocytes are capable of synthesizing new granules (Coles, 1986). Monocytes enter the circulation as relatively immature cells and reach their full functional capacity only when they migrate into tissues, differentiate into macrophages and enter areas of inflammation (Delder, 1998). Circulatory monocytes and tissue macrophages known as the mononuclear phagocyte system (MPS) play an important role in phagocytising and destroying intra-cellular organisms (Fungi, protozoa and viruses) transformed cells and cell debris. The MPS is also involved in regulation of granulopoiesis and erythropoiesis, and these cells have been reported also to have surface receptors for all immunoglobulins (Deldar, 1998).

2.17.5 INTERPRETATION OF LEUKOCYTE COUNTS

Accurate interpretation of leukocyte alterations is dependent upon an understanding of the various factors that influence either the total or differential leukocyte counts in both healthy or disease condiions. The physiological factors to be considered in the interpretation of leukocyte counts which was dependent on age, sex, temperature, nutritional status and degree of stress have been reported. Although the differential leukocyte count is usually reported in percentage, interpretation to any alteration should be based on the absolute numbers of the various cell types. If interpretation is based solely on the differential count with no consideration for the total count, erroneous conclusions may be reached (Coles, 1986).

(a) Leukocytosis - Leukocytosis is an increase in total leukocyte counts above the upper limit for the animal species. This increase in total leukocyte count is usually a consequence of an increase in the total number of circulating neutrophils although in some circumstances other cell types may also be increased. This alternation in the

leukocyte picture can be the consequence of a normal physiological response or disease condition. Pathologic leukocytosis as a rule is an increase in segmented neutrophilic granulocytes. This increase in neutrophils may be relative (an increase in the percentage of neutrophils) or absolute an (increase in the total number of cells) or both. Alterations observed according to Coles (1986) are for the most part a reflection of animal species response to disease.

(b) Leukopenia – Coles (1986) stated that leukopenia is a reduction in the leukocyte count below normal values which may be either balance in which there is a decrease in all cellular elements, or it may be confined to a single cellular element and is then referred to by the more specifc name – neutropenia, lymphopenia or eosionopenia. The general causes of leukopenia are related to alterations in the bone narrow and are known as the four D's-degeneration, depression, depletion and destruction. If any of these alterations occur in the bone marrow, the number of leukopenia appearing in the peripheral circulation is decreased.

2.18 SERUM BIOCHEMICAL PARAMETERS IN CHICKENS

When blood clot in a test tube, a solid red mass is formed. However, on standing longer, the clot will contract, expressing out a supernatant yellow fluid, which is called serum. Essentially serum is plasma minus fibrinogen and most clotting factors. The fact that serum contains antibodies that the animal may have formed makes it useful in prevention and treatment of disease.

2.18.1 Importance of Serum Indices

The serum chemical values are used to assess nutritional status of animals and also comparison of blood chemistry profiles with nutrients upward or downward for different population group (Kerr *et al.* 1982, Church *et al.*, 1984). They asserted that although nutrient levels in blood and body fluids may not be valid indications of nutrient functions of cellular levels, they are considered to be proximate measure of long term nutritional status. Biochemical indices also reflect circadium rhythms, gender age and hormonal changes and organ's diseases which influence the absorption, transport, metabolism or excretion of nutrient (Abubakar, 1997). The blood contains a myriad of metabolites and other constituents which provide a valuable medium for clinical investigation and

nutritional status of human beings and animals. Hence, Coles (1986) recommended the use of blood and biochemical parameters in medical nutritional assessment.

Church *et al.* (1984) reported the dietary components have measurable effects on blood components; hence blood constituents are widely used in nutritional evaluation and survey of animals. Serum or plasma chemical values are useful in the assessment of the nutritional and health status of human beings and animals. They may also indicate the adjustment of certain nutrient upward or downward. Biochemical values measured include serum total protein, albumin, globulin, albumin-globulin ratio, cholesterol, urea, creatinine, glucose, mineral and liver enzymes (Onifade *et al.*, 1993, Olorede *et al.*, 1995).

2.18.1.1 Protein Constituents

Proteins are complex organic compounds of high molecular weights which contain carbon, hydrogen, nitrogen and sulphur. Some contain other elements like phosphorus, iron, copper and zinc (MCdonald *et al.*, 1995). It is the nutrient found in highest concentration other than water in organs and muscle tissue. The proteins in the serum are referred to as serum protein, with different functions. The chemistry of serum is routinely used for the detection of organ disease in domestic animals. Generally, serum protein function is in defense mechanism i.e. the response of immunoglobulin to infection. They are also involved in the maintenance of plasma osmotic pressure. The serum proteins include the total protein, albumin and globulins.

2.18.1.2 Total Serum Proteins

The total serum proteins as its name implies represents the sum total of numerous different proteins, many of which vary independently of each other. Total protein is composed of the albumin and globulin. Ross *et al.* (1978) observed that total protein increases with age. Treacher (1977) stated that changes in the nutritional status are more easily reflected in albumin rather than globulin fractions of the blood. The total protein concentration must be measured when performing an electrophoresis in order to calculate the concentration of each protein fraction from its percentage. Aside from this situation, the determination of total protein supplies limited information except in conditions relating to changes in plasma or fluid volume such as shock dehydration, possible

overhydration and haemorrhage. The need for fluid is revealed by an elevated serum protein concentration that shows haemoconcentration. It is also useful to measure the total serum protein when determining the calcium concentration because the non diffusible calcium fraction is bound to protein and varies directly as the serum protein. Analyses for proteins are usually performed on serum because this is the fluid medium generally used in the chemistry laboratory.

2.18.1.3 Significance of Serum Proteins

The serum proteins have many functions that may be summarized as follows according to Kaplan and Szabo (1983):

(1) They affect the distribution of extracellular fluid between the vascular bed and interstitial fluid by means of the oncotic pressure they generate. This is a general property of proteins, but the most important protein in this respect is albumin because of its relatively small size and high concentration about 60% of the total plasma proteins.

(2) They serve as carriers for various cations and some compounds that are relatively insoluble in water such as bilirubin, fatty acids, steroid, hormones and lipids.

(3) They function as antibodies to provide a defense system for the body against foreign proteins, viruses and bacteria. This role is reserved for the gamma globulins.

(4) They form part of the endocrine system.

(5) They protect against damage to the vascular system by forming a complex blood clotting system.

(6) They provide tissues with a source of nutrients for building materials or calories

(7) Some proteins functions as enzymes.

2.18.1.4 Dietary effects on Total protein and Fractions

Latner (1975) had earlier observed the nutritional status of individual with respect to proteins. Effect of dietary proteins is serious on the synthesis of serum protein. The direct effect is seen in terms of provision of raw materials for synthesis while the indirect effect is seen in deprivation of the liver. Protein-energy malnutrition depresses serum proteins

like albumin, transferrin, prealbumin etc. However there are nutritionally unrelated factors that affect their concentration (McLaren, 1988). The nutritional status of an animal has a marked effect on synthesis of plasma protein. Lack of dietary protein can invariably impaired protein availability which has its most marked effect on levels of gamma globulins and albumin. Excessive decrease is plasma albumin may lead to oedema. A decrease in gamma globulins may result in impaired resistance to infectious agents. Plasma proteins serve as sources of nutrition for tissues (McLaren, 1988). Animal protein in feed was however, found to favour the production of serum albumin and hence the ability of animals fed animal protein based-diet to endure disease stress more efficiently than others (Chaudhuri, 1976).

Serum protein changes are however, not consistent with differences in protein utilization or growth. Generally, serum protein functions are in defence mechanism-the response of immunoglobin to infection, they are also involved in the maintenance of plasma osmotic pressure. The recommended value for total protein and albumin include 5.20 to 6.90 g/dl/ and 2.1 to 3.45 g/dl respectively (Mitruka and Rawnsley, 1981). Globulins are an important fraction of the plasma proteins and are dividend into alpha-1, alpha-2, beta and gamma globulin based on their different molecular weights.

The nutritive state may be dependent not only on the proper and adequate intake of protein materials or protein building materials in the diet, but may also be a reflection of the physiological state existing within the animal body reflecting alterations in metabolism. Alterations in plasma protein concentration existing within an animal may be indicative of a disease. The functional state of the liver and kidneys are important in internal metabolism of proteins. Drastic alteration in plasma protein values are often observed in association with body kidney and liver disease (Coles, 1986). Tumbleson *et al.* (1976) reported that the liver synthesizes all the components of serum total protein except the immunoglobulins which are produced by the spleen. A reduction in the concentration of these is an indication of liver dysfunction.

Serum total proteins and albumin synthesis are related to the amount of available proteins (Hoffenberg *et al*., 1966; Iyayi and Tewe, 1998) and not to the amount of calories. Similarly Eggum (1976) and Tewe (1985) reported a positive linear correlation between protein quality and total serum protein concentration and a negative correlation between

serum urea and creatinine concentration. Hypoproteinemia can occur with chronic renal or hepatic disease, malnutrition, malabsorption (e.g. intestinal parasitism) or chronic blood loss (Coles, 1986). Elevated total protein occurs with dehydration or if there is an increase in globulins. When serum proteins are fractionated on cellulose acetate, five bands are usually obtained at the end of the electrophoretic run when the support medium is stained with a dye that adsorbs to protein (Kaplan and Szabo, 1983). The bands are known as albumin, alpha one (α_1); alpha two (α_2), beta (β), and gamma (γ) globulins respectively, starting with the greatest migration toward the anode.

2.18.1.5 Serum Albumin

Albumin is one of the numerous different protein found in the serum. It is the largest individual protein fraction in avian plasma comprising 60% of the total serum protein (TSP) concentration (Altman, 1979; Coles, 1986).). It accounts for 35 to 50% of total protein (Bush, 1991). The albumin component of the serum has been reported to be in a physiological state of the equilibrium and falls only in a pathological state (Harper *et al*., 1979). Ross *et al.* (1978) confirmed that the most sensitive biochemical index of mild or impeding protein deficiency is a drop in serum albumin into the marginal range. It is synthesized exclusively in the liver from amino-acids, functions as a regulator of blood osmotic pressure, as a carrier for many cations and water insoluble substances (calcium, bilirubin, fatty acids) and as a pool of amino acids for caloric or synthetic purposes. It has a half life in plasma of about 17 days, which means that its plasma concentration would fall about 3% per day if synthesis were completely halted.

Hepatocellular damage usually results in a decrease in the serum albumin concentration but the change is relatively slow. The concentration of serum albumin is decreased in the following situation: Decreased synthesis; whether caused by: (a) Damage hepatic cells (b) deficient protein intake as in malnutrition and or starvation or (c) impaired digestion or absorption of protein products.

Extensive protein loss; whether (a) through the kidneys, as in the nephritic syndrome, (b) through the skin, following extensive burns or severe skin lesions as in exfoliative dermatitis or (c) through the gastro intestinal tract as in protein-losing enteropathies (protein-losing intestinal diseases).

Altman (1979) reported that a reading of total serum albumin than the normal physiological value usually indicates hypoalbuminenia which may result from; deficient intake of protein, deficient synthesis of albumin, excess protein breakdown, chronic liver disease, starvation and chronic gastrointestinal disease with their interference with protein digestion and absorption. Coles (1986) reported that albumin helps in detoxifying and inactivating toxic materials in an animal's body and is essential in the transport of fatly acids. Hyperalbuminemia, increase in serum albumin levels is said to be due to increase in total protein volume and are rarely seen except in cases of dehydration and shock. Generally speaking increases in albumin are masked by increase in total plasma volume,

Albumin exerts through its high osmotic properties more influence on plasma volume than any of the plasma proteins. Decrease in total serum albumin may result from deficient intake of protein, deficient synthesis of albumin, excess protein breakdown, chronic liver diseases, starvation and chronic gastro-intestinal diseases with the interference with protein digestion and absorption. Some of the biological functions of albumin are to maintain the water balance in serum and plasma, transport and store a wide variety of glands for example, fatty acids, calcium, bilirubin and hormones such as thyroxine, and provides an endogenous source of amino acids, a fall in serum albumin levels (hypo albuminemia) is said to be due to excess breakdown of serum protein levels due to diabetes mellitus, trauma and prolonged fever and sometimes impaired digestion of protein and lack of adequate absorption of protein /amino acids. This has been implicated in the characteristics diagnostic and prognostic signs in liver disease refer to as cirrhosis (Coles 1986). McLaren (1988) reported that decrease in total protein level is usually associated with low albumin levels, which are usually accompanied by a lesser change in globulins and low albumin-globulin ratio. Low albumin levels may be due to significant loss of albumin in the urine, decreased formation in the liver or insufficient protein level. Synthesized in the liver and has a half-life of 2 to 3 weeks. Increase in total protein is found in haemoconcentration due to dehydration from extreme loss of fluid from vomiting and diarrhea (Aniket, 2005).

2.18.1.6 Serum Globulins

Serum globulins are an important fraction of the plasma protein which is insoluble in bases, acids, and salt solutions (Coles 1986). The globulins are classified as alpha-1

(α1), alpha-2 (α 2), Beta-globulin (β-globulin) and Gamma globulin (γ-globulin) based on their different molecular weights and according to their motility in an electric field:

α_1 – Globulin

α_1 -globulin is a mixture of many proteins, some of which have been identified and characterized. The α_1-globulin band increases as a non specific response to inflammation arising from a variety of causes: infection, trauma and neoplasms. They are synthesized in the liver (Kaplan and Szabo, 1983).

α_2- Globulin

Some of the well-known proteins in this fraction include the haptoglobulins and ceruloplasmin, a copper binding protein that has oxidase activity. The microproteins in the α_2-fraction also are increased in inflammatory conditions (Kaplan and Szabo, 1983).

β-Globulin

The β-Globulin fraction consist the β lipoproteins, the iron-transporting protein (transferrin), florinogen and other lesser known proteins. Any condition that increases the β-lipoproteins makes this band more prominent because the lipoproteins predominate in this fraction (Kaplan and Szabo, 1983)

γ- GLOBULIN.

The globulins are involved in the various immunological responses. The immunoglobulins play a regulatory role in autoimmune diseases (diseases caused by antibodies to substances natural to the body) (Aniket, 2005). Elevated increase in globulin may be as a result of hepatic disease, cirrhosis of the liver, biliary cirrhosis, lympho proliferation disease and gemo chromatosis (Murrary *et al.*, 1988). Serum globulin is influenced by types of dietary protein. Coles (1986) asserted that hyper-glubulinemia is often accompanied by a decrease in total albumin and this is due to an alteration in total protein. This situation often leads to hypo-proteinemia. High levels of serum globulin are often due to liver infections. Hypoproteinemia is usually associated with bad diets and or

poor absorption of nutrients from the gastro-intestinal tract. In cases of hepatitis and fibrosis, the serum globulin levels are very high.

2.18.1.7 Albumin-Globulin Ratio

Chauduri (1976) reported that diet especially protein, has a profound influence on the generation of the drift of serum proteins. Stress from disease has been reported to alter serum protein pattern by causing sharp fall in albumin/globulin ratio i.e. the percentage globulin increased because antibodies are located in the globulin (Salako, 2001). Injuries or infections can increase globulin proteins while decreasing albumin leaving the total serum protein values within the normal range even though a problem exists (Coles, 1986). A decrease in the total protein level is usually associated with low albumin levels which are usually accompanied by lesser damage in globulins and in low Albumin-Globulin ratio. Low albumin levels may be due to significant loss of albumin in the urine, decreased formation in the liver or insufficient protein levels.

In oedema of nephritic origin, the albumin is decreased significantly as is the Albumin-Globulin ratio (Nissi, 2004). Globulins may be lost or decreased with albumin when protein synthesis is deficient in cases of malnutrition and malabsorption, but the Albumin-Globulin ratio is usually normal (Duncan *et al.,* 1994). If albumin is selectively lost or deficient, the albumin-globulin ratio will be low but if there is a concomitant loss or failure to synthesize globulins, panhypoproteinemia and a normal Albumin-Globulin ratio may occur (Duncan *et al.*, 1994). In case of hypoglobulinemia, globulins are selectively reduced and a high Albumin-Globulin ratio occurs. Olayemi *et al.* (2002) reported the AST (i.u/l), Total Protein (g/dl), albumin (g/dl) and globulin (g/dl) of adult Nigerian duck as 29.93 ± 5.06, 5.91 ± 0.29, 2.81 ± 0.21, 3.09 ± 0.11 respectively. He also reported that in the domestic fowl, the total protein value decreased with age. Oyewale *et al.* (1988) reported that in the domestic fowl, globulin value was higher, albumin and total protein value lower than those obtained for the Nigerian duck. Taiwo and Ogunsanmi (2003) reported values of total protein, albumin, globulin and AST of the West Africa Dwarf goat as 7.8 ± 0.3 g/dl 3.8 ± 0.29 g/dl, 4.0 ± 0.3 g/dl and 75.8 ± 3.3 iu/L respectively. Mitruka and Rawnsley (1981) reported the mean normal values of cholesterol and glucose of the cockerel as 100mg/dl and 162mg/dl respectively.

2.18.2 Serum Enzyme Activity

Tests dependent upon the measurement of serum enzyme activities is one of the categories of tests employed in the examination of hepatic cell damage (Bush, 1991). Alterations in serum enzyme activities due to malfunctioning of the liver occur as a result of these processes (Coles, 1986). However, two of the processes are emphasized in this study; they are:

(1) Those who involve elevation of enzymes due to disruption of hepatic cells as a result of necrosis or as a consequence of altered membrane permeability. Included in this group are the enzymes Alanine aminotransferase (ALT), formerly known as glutamic pyruvic transaminase (GPT) and Aspartate aminotransferase (AST) formerly called glutamic oxaloacetic transaminase (GOT).

(2) Those who involve elevation in enzyme levels due to the lack of biliary excretion as seen in obstructive icterus (Alkaline phosphatase).

The aminotransferases (ALT and AST) have a wide distribution in animal tissues and are present in small quantities in the serum of all animals as a consequence of normal tissue destruction and subsequent enzyme release. Since these enzymes have their principal functions within the cell, increases observed in the serum are often a reflection of cellular destruction or disease.

(1) Alanine aminotransferase (ALT)

Alanine aminotransferase also called GPT is present in many tissues. The enzyme catalyses the transfer of the amino group of alanine to α ketoglutarate, resulting in the formation of pyruvate and glutamate.

Alanine + α-oxoglutarate $\rightarrow$ pyruvate and glutamate

This enzyme may be assayed by coupling via the product, pyruvate to an LDH –catalysed indicator reaction. Alanine aminotransferase is found in high concentration in liver cells and enzyme is released into the plasma by liver cell death, a normal event. However, when liver cell death increases, ALT levels rise above the normal range. The spill over of the enzyme into blood is routinely measured as a marker of abnormal liver cell damage; hence markedly raised plasma activity indicates a severe liver diseases usually viral hepatitis or toxic liver necrosis (Champe and Harvey, 1994). An elevated ALT in the

presence of normal levels of plasma alkaline prosphatase helps distinguish liver disease caused by liver cell damage from diseases caused by problems in biliary ducts.

(ii) Aspartate aminotransferase (AST)

Aspartate aminotransferase, formerly known as glutamate oxaloacetate transaminase (GOT), an enzyme widely distributed throughout the body catalyses the reaction:

Aspartate + α-oxoglutarate → oxaloacetate + glutamate

It may be assayed by coupling, via the product, oxaloacetate to an indicator reaction catalysed by malate dehydrogenase which involves NAD^+/NADH as coenzyme: AST is an exception to the rule that aminotransferase turned amino group to form glutamate. During amino acid catabolism, aspartate aminotransferase transfers amino group from glutamate to oxaloacetate, forming aspartate which itself is used as a source of nitrogen in the urea cycle. AST is present in all tissues of the body and is not an organ specific test and consequently may be utilized to detect destruction in a wide variety of tissues. As this enzyme appears in extremely high concentration in muscle, both skeletal and cardiac, it is of value in confirming a diagnosis of muscular degeneration. Markedly raised plasma activities (10-100 times normal) of AST usually indicate severe damage to the cells of the heart (as in myocardial infarction) or liver (as in viral hepatitis or toxic liver necrosis). Moderate increases of activity are found in many diseases Trevor, 2001).

iii) Alkaline Phosphatase

Phosphatases are agents which hydrolyse esters with the liberation of inorganic phosphate. Alkaline phosphatases are widely distributed in body being high in the bone (Osteoblasts), intestinal mucosa, renal tubules cells liver and placenta (Coles, 1986: Bush 1991). Increase in the serum level of ALP gives an indication of liver insufficiency or obstructive icters (Bush, 1991). It has a pH optimum between 9 and 10. Determination of serum alkaline phosphatase may be of value in assaying bone abnormalities and is of significance in the study of hepatitis diseases. Increased serum at alkaline phosphatase activity has been reported to occur in dogs following experimental obstruction of the bile duct. Although alkaline phosphatase activity also increases in hepatocellular damages, the level that occurs during obstructive icterus (bone abnormality) is usually much higher (Coles, 1986).

ALP is produced primarily in the liver and in the bone. It's also produced by the placenta of a pregnant female and, to a lesser extent, by the intestines and kidney (Nissi, 2004).In the same vein, Aniket (2005) stated that alkaline phophatases are a group of enzymes primarily in the liver (Isoenzyme ALP-1) and bone (isoenzyme ALP-2). There are also small amounts produced by cells lining the intestines (isoenzyme ALP-3), the placenta, and the kidney (in the proximal convoluted tubules). What is measured in the blood is the total amount of alkaline phosphates released from these tissues into the blood. As the name implies, this enzyme works best at an alkaline pH (a pH of 10), and thus the enzyme itself is inactive in the blood. Alkaline phosphates act by splitting off phosphorus (in an acidic medium) creating an alkaline pH.

The primary importance of measuring alkaline phosphate is to check the possibility of bone disease or liver disease. Since the mucosal cells that line the bile system of the liver are the source of alkaline phosphates, the free flow of bile through the liver and down into the biliary tract and gallbladders are responsible for maintaining the proper level of this enzyme in the blood. When the liver, bile ducts or gallbladder system are not functioning properly or blocked, this enzyme is not excreted through the bile and alkaline phosphostase is released into the blood stream. Thus the serum alkaline phophostase is a measure of integrity of the hepatobiliay system and the flow of bile into the small intestine (Nissi, 2004). Alkaline phosphotase (ALP) is an enzyme in the cells lining the biliary ducts of the liver. If there is an obstruction to the bile diet (choleslasis) e.g gallstones, ALP levels in plasma will rise. ALP is also present in bone and placental tissue, so it is higher in growing animals as their bones are being remodelled (Wikipedia, 2002).

Nissi (2004) stated that, normally the liver produces more ALP than other organs or the bones. Some conditions can release large amounts of ALP into the bloods stream. The conditions include rapid bone growth (during puberty) bone disease (osteomalacia or paget's disease) or damaged liver cells. Toro and Ackerman (1975) stated that ALP composed of several isoenzymes. Electrophoresis, starch, agar gel or acrylamide gels have been used to fractionate ALP isoenzymes. ALP originating in the bones migrates more slowly than that originating in the liver and this can be used to distinguish between elevation in total ALP due to liver disease and bone disorders. The recommended value for ALP is 24.5 to 44.4. iu/l (Mitruka and Rawnsley 1981).

2.19.2.1 Mechanisms of action of aminotransferases

Aminotransferases are a family of enzymes, which catalyses the transfer of amino groups from one carbon skeleton to another. All amino acids with the exception of lysine and threonine, participate in transamination at some point in their catabolism. All aminotransferases require pyridoxal phosphate (a derivative of vitamin B_6), which is covalently linked to three-amino groups of a specific lysine residue at the active site of the enzyme. They act by transferring the amino group of an amino acid to the pyridoxal part of the coenzyme to generate pyridoxamine phosphate. The pyridoxamine form of the coenzyme then reacts with an α-keto acid to form an amino-acid and regenerates the original aldehyde form of the coenzyme. These two most important aminotranferase reactions are catalysed by alanine aminotransferase and aspartate aminotransferase.

2.18.3 SERUM LIPIDS

2.18.3.1 Cholesterol and Triglycerides

The lipids are fats and fatty acid derivatives together with some other substances of similar solubility properties that are concerned with fat metabolism. Reference is sometimes made to the blood lipids, but usually the substances determined are contained in serum (or plasma). There are some lipids in the erythrocytes (chiefly in the cell membrane) but these are not concerned with fat metabolism. The main constituents of total serum lipids are cholesterol and cholesterol esters, phospholipids and triglycerides. There are also small amounts of free (unesterified) fatty acids, mono and di-glycerides and other steroids together with the fat-soluble vitamins. The lipids are relatively insoluble in aqueous solution and are present in serum either in combination with certain protein (lipoproteins) or in an emulsified form (chylomicrons).

Serum cholesterol is one of the lipid fractions of blood. It is known to be regulated by such factors as species, age, and sex in particular diets (Babatunde *et al.*, 1987). High fat and low dietary protein are known to cause increase in serum cholesterol. Low blood cholesterol may be due to unsaturated fatty acid in diet. Morag (1989) stated that high level of dietary fat increases the amount of cholesterol absorbed in the blood. Cholesterol is a fat-like substance in the blood much, also if elevated has been associated with heart disease. Cholesterol in itself is not all bad; in fact, the body needs a certain amount of this

substance to function properly. However, when the level gets too high, vascular disease can result. As the level of blood cholesterol increases, so does the possibility of plugging the arteries due to cholesterol plaque buildup. Such a disease process is called "hardening of the arteries" or atherosclerosis. When the arteries feeding the heart become plugged, a heart attack may occur. If the arteries that go to the brain are affected then the result is a stroke.

These are three major kinds of cholesterol, High Density Lipoprotein (HDL) Low Density Lipoproteins (LDL) and Very Low Density Lipoprotein (VLDL). LDL cholesterol is considered "bad cholesterol" because cholesterol deposits form in the arteries when LDL levels are high. HDL – Cholesterol is "good cholesterol" as it protects against heart disease by helping to remove excess cholesterol deposited in the arteries. High levels seem to be associated with low incidence of coronary heart disease. The range of concentration of cholesterol for normal male and female chickens is 52.0 – 148.0 mg/dl (Mitruka and Rawnsley, 1981).

Triglycerides - these are the chemical forms in which most fat exists in the body, as well as in food. They are present in blood plasma. Triglycerides, in association with cholesterol, form the plasma lipids (blood fat). Triglycerides in plasma originate either from fats in our food, or are made in the body from other energy sources, such as carbohydrates. Calories we consume but are not used immediately by our tissues are converted into triglycerides and stored in fat cells. When your body needs energy and there is no food as an energy source, triglycerides will be released from fat cells and used as energy - hormones control this process.

2.18.4 SERUM CHEMICAL COMPONENTS

2.18.4.1 Serum glucose

The glucose level is used to sustain normal function of blood. The level of blood glucose depends on the balance between intake of carbohydrate and endogenous glucose synthesis and release by the liver on one hand and glucose storage, utilization and excretion on the other hand. The response of an individual to carbohydrate load is glucose tolerance. Impaired glucose tolerance occurs when blood/plasma glucose level rise high and the rise is more prolonged than in the normal situations. The recommended value is 152.0 to 182.0 g/dl in chickens (Mitruka and Rawnsley 1981). Glucose is continuously required as

an energy source by all the body cells and therefore it is essential to maintain adequate level in the plasma (Dukes, 1975). Serum glucose is affected by numerous factors. Increased levels are associated with 'Diabetes mellitus', hyperactivity of the thyroid, pituitary or adrenal glands. Decreased levels are observed in cases of insulin over dosage, insulin secreting tumours, myxodemia, hypopituitarism, hypoadrenalism and conditions interfering with glucose absorption (Sigma, 1991).

2.18.4.2 Serum urea nitrogen

Urea is formed in the liver and represents the principal end products of protein catabolism. Urea normally has no useful function in the body other than a possible mild diuretic action and is excreted almost entirely by the kidneys. Urea in plasma is filtered by the glomerulus and under normal conditions approximately 25 to 40 percent of filtered urea is reabsorbed as it passes through the tubules (Coles, 1986). Urine flow rates that are greater than normal diminish tubular reabsorption and conversely low rates of urine flow increase urea reabsorption in the tubules.

Amino-acids imbalance of the diet can cause increased blood urea concentration and the restoration of normal level is achieved by feeding a diet having balance amino acid level. There is a high negative correlation between the biological value of the dietary protein and blood urea. This is consistent with the demonstration that increases in dietary protein content causes decrease in biological values (Eggum, 1980). Blood urea concentration depends on quality and quantity of protein in the diet and the time of sampling. There was however no relationship between the live weight of the animal and blood urea concentration.

High urea level indicates a low protein quality being fed and in cases where energy deficiency or diseases prevent efficient utilization of protein, then serum urea levels are high as a consequence of the increased deamination of immetabolised amino acid in the liver. Diets high in protein elevate serum uric acid levels due to increase in uric acid biosynthesis. Imbalance of diet can cause an increased blood urea concentration which can be restored upon feeding a balanced amino acid diet (Latner, 1975). He reported that high blood urea nitrogen indicates that the animal is on high protein diet plasma urea nitrogen related to the intensity of nitrogen metabolism and depends on quantity and quality of dietary protein. Plasma urea increase with age and its concentration is higher on a high protein diet than on a low protein diet (Baron 1982). Plasma urea nitrogen had

been reported to give values approximating twice that of blood urea 1mg /L urea= 2.14 mg/L blood urea nitrogen (Hewitt *et al.*, 1989).

The serum urea nitrogen test measures the amount of nitrogen contained in the urea. High serum nitrogen levels can indicate kidney dysfunction, but because blood urea nitrogen is also affected by protein intake and liver function, the test is usually done in conjunction with a blood creatinine, a better indicator of kidney function. Increased blood urea level is always associated with chronic generalized nephritis (Kidney damage) (Coles, 1986) and increase in dietary protein level. The status of protein metabolism within the body regardless of diet may also influence blood urea nitrogen concentration. Degeneration of the tissues as a consequence of fever, trauma, infection or toxemia may result in an increase in blood urea concentration. A similar increase may be seen in association with haemorrhage into the gastrointestinal tract. Generally any factors that decreases glomerular filtration rate (GFR) will increase serum urea concentration. Mitruka and Rawsley (1981) gave a range of 1.50-6.30mg/dl as the serum urea concentration in normal male and female chickens.

2.18.4.3 Creatinine

Creatinine is a non protein nitrogenerous substance and a metabolic by product of muscle energy metabolism of creatinine and phosphocreatinine. Creatine is important in muscle metabolism and its metabolic end product, creatinine, is found in muscle and blood, it is used to measure damage of tissue catabolism because it is proportional to muscle mass (Baron, 1982). Serum creatinine is usually relatively constant and it is not indicative of diet utilization. Production of creatinine depends on an individual's muscle mass which usually fluctuates very little. It is excreted by glomerular filtration and significant quantities are neither excreted nor reabsorbed by the tubules. If production remains constant, the measurement of serum creatinine may provide a crude index for glomerular filtration. Factors such as diet, fever, toxemia, infection and drugs do not as readily influence creatinine levels. Also, daily production of creatinine from muscle metabolism is relatively constant (Coles, 1986).

The rate of excretion is influenced by glomerular filtration rate and any abnormality that decreases it will result in an increase in the concentration of serum creatinine. As there

are fewer non-renal factors that may influence creatinine it has had a reputation of being a more specific test for the diagnosis and prognosis of progressive renal disease. Osborne *et al.* (1972) reported a marked elevation in serum creatinine concentration indicates severe functional or organic impairment of nephron function. However, serum uric acid level is indicative of N-retention, increased urea, creatinine and other-protein constituents. Eggum (1980) reiterated that serum creatinine indicates the extent of muscle wastage and a high level shows a high degradation of muscle phosphocreatine to form creatinine. A linear relationship between protein and total serum indicating that creatinine can be used as an indirect measure of protein quality was reported. The serum creatinine concentration range for normal male and female chickens is 0.90-1.85mg/dl (Mitruka and Rawsley 1981).

2.18.4.3.1 CREATININE AND ITS CONCENTRATION

Creatinine is a breakdown product of creatine, which is on important component of muscle and part of the cycle that produces energy needed to contract the animal muscles. Creatinine could also be referred to as protein produced by muscle and released into the blood. Creatinine can be converted to the ATP molecule, which is a high energy source. The daily production of creatine and subsequently creatinine depends on muscle mass, which fluctuates very little. Therefore, creatinine concentration will be slightly higher in male chickens than in female chickens. Creatinine is excreted from the body entirely by the kidneys with normal renal excretory functions; the serum creatinine level should remain constant and normal. Blood levels are a good measure of how well the kidneys are working (Eggum, 1980).

2.19.4.3.2 SERUM CREATININE TEST

A serum creatinine test measures the amount of creatinine in the blood. The test is performed to evaluate kidney function. If kidney function is abnormal, creatinine level will increase in the blood, due to decreased excretioin of creatinine in the urine. The creatinine level in the serum is therefore determined by the rate of its removal, which is roughly a measure of kidney function. If kidney function falls (for instance, a kidney is removed to donate to a relative) the creatinine level will rise (Eggum, 1980).

2.18.4.3.3 EFFECTS OF CHANGES IN CREATININE LEVEL IN THE BLOOD

Increased creatinine levels in the blood suggest diseases or conditions that affect kidney function. They include; damage to or swelling of blood vessels in the kidney (glomerulonephritis) caused by infection or auto immune diseases; bacterial infection of the kidneys (pyelonephitis), death of cells in the kidneys' small tubes (acute tubular necrosis) caused by drugs or toxins, prostate diseases kidney stone, or other causes or urinary tract obstruction or reduced blood flow to the kidney due to shock, dehydration, congestive heart failure, arterosclerosis, or complications of diabetes. Creatinine can also be increased temporary as a result of muscle injury. Low levels of creatinine are not common and are not usually a cause of concern. They can be seen with conditions that result in decreased muscle mass. Creatinine levels are generally slightly lower during pregnancy (Baron, 1982). The serum creatinine concentration range for normal male and female chickens is 0.90-1.85 mg/dl (Mitruka and Rawnsley, 1981)

2.19 FACTORS AFFECTING BLOOD PARAMETERS

Sex: This has an effect on RBC, PCV and Hb. Orji *et al.* (1987) reported that male guinea fowl has higher value than females. This has been attributed to the role of testosterone (Oyewale and Ogwuegbu, 1986)

Age: Sarror and Vanveen (1977) reported that erythrocytes, haemoglobin and PCV increase with age of large white pigs and the values becomes stabilized at 30 days of age.

Breed: Chiboka and Thomas (1981) reported that breed or species of animals affect blood parameters.

Diseases: Bains (1979) reported that disease like coccidiosis, avian eucophalanyento etc affect blood parameters. It was earlier reported by Sarror & Vanveen (1977) the values of Hb, RBC, PCV and WBC of healthy animals are different from those from unhealthy animals. They concluded that haematological values from clinically healthy flock could serve as a baseline for interpreting blood data.

Nutrition: Blood parameters can be affected by nutritional deficiency and type of feed offered to animals (Kerr, 1979).

2.20 ORGANS AND THEIR FUNCTIONS

For the purpose of this study, the organs of interest are briefly discussed here with respect to their various functions in the cocks.

(i) Liver

This is one of the most complex organs in this animal. It serves as the body's main chemical factory and one of the major storehouses of food. A reddish brown mass, it lies in the upper right part of the abdomen directly under the diaphragm and above the stomach and intestines. The liver function in diverse ways, digestion and use of food, secretion of bile, regulates nutrients amount available to each cell, purifies the blood, manufactures various blood proteins e.g. albumin, globulins d fibrinogens (WBE, 1995).

(ii) Heart

The heart is a very important organ in the animal system. It powers the body, sends life giving blood throughout with the aid of pump actions. Together with the other tube -like structures that transports blood (arteries, veins and capillaries) they are called the cardiovascular system (WBE, 1995).

(iii) Lungs

This is the chief breathing organ and its main job deals with the exchange of gases. As blood flows through the lungs, it picks up oxygen from the air sac and releases carbon dioxide. The lungs help clean the blood of harmful substances by filtering bloods in the capillaries so that particles such as blood clots and fat globules are removed. (WBE, 1995).

(iv) Spleen

This is a soft, purplish organ located behind and to the left of the stomach in animals. It plays an important role is both the circulatory system and immune system. It is a spongy organ that filters foreign unwanted substances and damaged cells from the blood. The spleen also helps to fight infection from certain parasites and bacteria through its macrophages. It contains lumps of white blood cells called lymphocytes which release special protein into the blood. These proteins called antibodies weaken and kill bacteria, viruses and other substances that can cause injection (WBE, 1995)

(v) Kidney

These are two bean-shaped kidneys in the body of a mammal. These kidneys lie asymmetrically on the dorsal body wall of the lumbar region of the abdomen, the right kidney being more anterior than the left. Each kidney is reddish-brown in colour and consists of two distinct regions, an outer cortex and an inner medulla. The kidneys remove unwanted nitrogenous substances, e.g. urea and ammonium compounds and dissolved carbon dioxide from the blood. They get rid of excess water and salts and keep the osmotic concentration of blood constant and also maintain the acid base balance in the body (WBE, 1995).

2.21 TESTES OF COCKS.

A rooster (male chicken) does not have an external scrotum; however, he does have testes. Located high in the abdominal cavity in front of the kidney and near the backbone, the two bean-shaped testes consist of many slender ducts. The lining of these ducts produces sperm. The testes shrink and grow on a regular basis, becoming larger during active mating. Often the left testis is larger than the right one. In addition to creating sperm, the testes also produce the male hormones that influence mating, male behavior, the comb growth atop their heads, the size of the tail feathers, the spurs on the insides of their feet and the red wattles under their chins (McCartney, 1999).

2.22 CARCASS CHARACTERISTICS AND EVALUATION

The quality of carcass goes a long way in the determination of the profit obtained from the poultry enterprise. The plane of nutrition, sex, age, breed and management of the birds determines the carcass quality. Dzudie (1994) stated that for manufacturing and domestic consumption, the quantitative requirement in an animal carcass is best assessed when the proportion of muscles is at a maximum, fat at the optimum and bone is at a minimum.

(1) Meat Quality

Food and Agriculture Organisation of the United Nations (FAO, 2014) defined meat quality by the compositional quality (lean to fat ratio) and the palatability factors such as visual appearance, smell, firmness, juiceness, tenderness and flavor. Enfalt *et al*. (1997) stated that meat quality is often used when describing technical and sensory quality. It can also include other parameters such as carcass composition, ethical considerations,

production, economics, nutritional value and microbiological status. Bray *et al*. (1969) defined meat quality as a combination of traits that provides for an edible product which loses a minimum of nutrients, free of spoilage and other abnormalities after processing and storage. The meat product is appetizing, attractive, nutritive and palatable after cooking. Pearson (1994) considered the palatability of various meat under the following classification; colour, flavor, juiciness, tenderness, water binding properties, microbial problems, additives, residues and contributions of meat to human nutrition.

(ii) Colour of Meat

Colour is the primary sensory attributes of meat quality (Ikeme, 1990). One of the most important factors in the selection of meat product is colour (Hedrick *et al.*, 1994). Krop (1980) opinioned that the simple greatest factor determining the purchase of meat was probably the muscle colour. Meat should have a nirmal colour that is uniform throughout the entire cut (FAO, 2014). Tissues from older animal are darker in colour most times because muscle colour intensity increases with age. The colour of the muscle also depends on the part of the body the muscle was removed from and the type of animal e.g. beef is bright cherry red, chevron is light pink to red and chicken is white.

(iii) Meat Flavor

The desired flavor of meat is developed by the application of heat. Physiologically, the perception of flavor involves the detection of four basic sensations (salty, sweet, sour, and bitter) by nerve endings on the surface of the tongue, while aroma is detected when numerous volatile materials stimulate the nerve endings in the nasal passages (Forrest *et al.*, 1975). Flavour of cured meat is known to be different from that of the uncured one. Curing causes an increase in free amino acids, which is further enhanced on cooking (Balabukh and Lyaskonkoy 1980). Pre-slaughter factors affecting flavor of meat include breed, sex, age and feed. Others are stress at slaughter, post mortem, ageing and storage. Meat flavor is affected by type o species, diet, cooking method amd method of preservation (e.g smoked or cured) (FAO, 2014)

(iv) Juiciness of Meat

This is an impression of wetness produced by release of meat fluid. Forrest *et al.* (1975) and FAO (2014) established that the major contribution to the sensation of juiciness is the

water retained in the cooked product. Juiciness scores for cooked meat from different species of animals and from different cuts of meat vary greatly (Price and Schweigent, 1972). The sensation of juiciness in cooked meat is closely related to intramuscular fat content (Aduku and Olukosi, 1991) and any condition influencing intramuscular fat content of cooked meat will be reflected in the juiciness scores. Thus, the well-marbled meat from mature animal with a relatively high degree of finish is juicer than those from young animals with less marbling. Tumbling is suggested to positively influence tenderness and juiciness of meat as a result of the modification of its structure (Dzudie and Okunbanjo, 1999, Hulberg *et al.*, 2005).

(v) Tenderness and Texture

The overall impression of tenderness is obtained from the ease with which the teeth bits into the meat after chewing, the each with which meat creates fragments and the amount of residue remaining after chewing. Tenderness and texture are related. Tenderness usually refers to the ease of shearing or cutting during mastication whereas texture is primarily associated with greasiness, softness and structural fineness of the product before and after mastication. The large variability in meat tenderness as observed with identical muscle of homogeneous animal group originates from endogenous factors such as species, age, sex, breed, muscle location and environmental factors related to stress, slaughter, cooking condition and ageing (Smulders *et al.*, 1991; FAO 2014).

The less the connective tissue in meat, the more tender is the meat (Ihekeronye and Ngoddy, 1986). Boles and Shandi (2001) suggested that tenderness is fresh meat can be improved by hanging or the use of tenderizing agents such as potash, popain and bromelin juice that affects the structural and physiological conditions in muscle. Heating causes more or less extensive denaturing of the protein of muscle fibers, which reduces their water holding capacity and then the tissue shrinks, becomes harder and more compact, post-mortem storage of carcass at 0^{o}C to 2^{o}C for 7 to 21 days has been known to increase tenderness of beef. One important way to tenderize meat is by aging. Carcasses are aged by holding them at refrigeration temperature for extended periods of time after slaughter and initial chilling (FAO, 2014).

2.22 QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF COCKS SEMEN.

The qualitative and quantitative evaluation of semen of cocks enhances the assessment of its reproductive status. Several reports on semen characteristics of the domestic fowls have indicated that breed and strain significantly affects semen quality and quantity (Bah *et al*., 2001; Peters *et al*., 2008). Both qualitative are quantitative characteristics of semen have marked effects on fertility It is known that as cockerels become older they put on weight. From 40 – 45 weeks of age, their volume of semen declines and so does the frequency of mating, largely due to increasing incidence of foot problems. However as pullets get older they need a progressively greater volume of semen and a higher frequency of mating in order to maintain as acceptable level of fertility (Gaynor, 1986). Semen quantity is assessed in terms of its volume, sperm concentration and the number of spermatozoa per ejaculate (output) whether they are normal or abnormal and alive or dead. Semen quality is represented mainly in terms of sperm motility, viability morphology, semen pH, color and viscosity.

2.23.1 ASSESSING FERTILITY OF SEMEN

The Online Medical Dictionary described fertility as the capacity or ability to conceive or induce conception and thus generate or produce offspring. Fertility in males requires production of a sufficient number of mature and motile spermatozoa that can undergo capacitation and acrosome reaction to bind and penetrate the zona pellucida of the oocyte, which subsequently divide and forms a viable feotus. Defective sperm functions can lead to infertility in males. Sperm functions can be determined from assessment of semen parameters, which include sperm concentration, maturation, motility, capacitation, acrosome reaction, binding and penetration of zona pellucida (Naz and Minhas, 1995; Bah *et al*., 2001). Two parameters, namely, spermatozoa production and maturation contribute to male fertility. To achieve fertilization, spermatozoa need to complete the maturation process, which include epididymal maturation, capacitation in the female reproductive tract, and acrosome reaction at the fertilization site. Defects in any process can lead to spermatozoa infertility or subfertility.

2.24.2 SEMEN ANALYSIS

This is a standard starting point in assessment of semen quality. The parameters of the analysis composed of semen volume, sperm concentration, sperm density and spermatozoa motility, sperm morphology and acrosome evaluations. Several methods are used to assess semen quality in poultry and determine fertilizing ability. Some of these methods are regarded as highly subjective, while others require specific laboratory services Hazary and Wishart, (2001). In general, the quality assessments in semen have been the percentage to motile spermatozoa, percentage of morphologically normal spermatozoa and percentage of spermatozoa with normal acrosome and and seminal fluid characteristics (Woelders, 1991), DNA and lipid content of spermatozoa and ability to pass through a sephadex-glass wool filter (Saacke, 1982.) The most correlated parameter with fertilizing ability is the percentage of morphologically normal spermatozoa. However, the semen analysis is not a complete or accurate predictor of fertilization ability (Kruger *et al.,* 1986). Gebriel *et al*. (2009) reported that body weights of Norfa cocks had positive phenotypic correlations with semen volume, sperms concentration and live sperms, whereas, negative estimates were observed between body weight and abnormal sperms), semen pH) and sperm motility.

2.24.2.1 Semen volume

Semen volume varies with method of collection (Sturkie, 1970), feed (Mann, 1974), species breeds and live weight (Egbunike and Oluyemi., 1979, Moss *et al.,* 1979), frequency of collection (Pfaff *et al.,* 1986), season and age (Nwakalor, 1986). Sturkie (1970) reported that semen volume of cocks varies from 0.10ml to 1.00ml depending on the method of collection and breed. Some variations in semen volume are due to the amount of the seminal fluid from associate glands to the testes and will vary with the operator. A large volume of semen means increased seminal fluid with nutritive substance for sperm motility and viability which indicate a positive correlation between volume and motility. The volume of semen obtained from turkey (tom) at ejaculation is relatively small, varying from barely measurable amounts to slightly more than 0.3ml (Carson *et al.,* 1995). It was observed by LeDee *et al.* (1981) that chicken semen is smaller in volume than the average volume of 0.09ml reported for guinea fowl. Bah *et al*. (2001) reported semen volume of Sahel regional local breeding cocks to be averaged 0.28 mL.

Tuncer *et al*. (2006) reported semen volume of Denizli cocks to be 0.7 mLs. It also falls within the range reported by Peters *et al*. (2008) 0.37-0.73 mL, 0.76 mL for Nigerian indigenous breeds. Low volume may indicate partial or complete blockage of the seminal vesicles (Semen Analysis Wikipedia, 2007). The high semen volume and total sperm count is an indication of the superior genetic tendencies of Nigerian indigenous cocks for reproductive ability and higher fertility (Ajayi *et al*, 2011).

2.24.2.2 Spermatozoa Concentration

The actual number of spermatozoa in a given volume of an ejaculate, which gives a true picture of the fertilizing potential of the cock, is the sperm concentration. It gives a better view of a male animal than semen volume output (which is a function of semen volume and sperm concentration) when comparing semen from cocks of the same breed but placed on different dietary treatments. Sperm concentration has been determined by direct counts in the haemocytometer (Saied and Al – Soudi, 1975) or by measurement of the optical density of the semen sample (Brillard and Mc Daniel, 1986) or by measuring with an Evans Electro–selenium Ltd colorimeter calibrated against haemocytometer count. Bah *et al.* (2001) and Peter *et al*. (2008) reported sperm concentration 2.26 ± 1.08 x 10^9 sperm/ml of local breeder cocks and 3.52±1.00 x 10^9 sperm/ml for white leghorn respectively. 0.027 x 10^9 and 0.259 x 10^9/ml has been reported for guinea fowl and domestic chickens respectively by LeDee *et al.* (1981). Van Duijn (1984) estimated the number of spermatozoa needed per insemination to maintain a high level of fertility in chickens to be 62 million. However lbe and Obi (1986) have reported that a lower value of 60 million sperm cells could also give optimum fertility. Wentworth and Mellen (1963) reported the recommended amount of whole semen for insemination into hen as 0.05ml and this is expected to provide approximately 175 million spermatozoa. They emphasized that the 0.05ml dosage provides a safety factor under ordinary condition while Ambula (2014) recommended 0.1ml for the volume of semen required per hen during insemination. It is reported that sperm concentration is positively related to the colour (Jamudeen *et al.*, 1982) and negatively related to sperm abnormality. It is unrelated to semen volume and progressive motility (Egbunike and Oluyemi, 1979).

2.24.2.3 Spermatozoa Morphology

Morphology is a predictor of success in fertilizing oocytes during in vitro fertilization. Usually sperms abnormalities indicate disturbances of spermatogenesis and this could be attributed to age, nutrition and pollution (Bah *et al*., 2001) Abnormal sperm cells are contained in each ejaculate of an animal. Such abnormal sperm cells are not usually associated with lowered fertility until the proportion of abnormal sperm cells in an ejaculate exceeds 10% (Semen Analysis Wikipedia, 2007). Sperm morphology was recommended to be one of the most essential qualitative characteristics of poultry semen. It could be used as an essential parameter for predicting the fertilizing ability of spermatozoa (Kuster, 2004). The morphology of the chicken spermatozoa indicates that they are filiform in shape and nearly indistinguishable by light microscopy. Generally the head is slightly curved and consists of the acrosome and nucleus. The tail consists of the neck, midpiece and principal piece. The primary adnormalities is an aberration in the process of spermatogenesis due to disturbances by congenital or hereditary factors for example rudimentary tails, twin head and small head. Secondary abnormalities arise as a result of a fundamental problem with the process of maturation stage of spermatogenesis where abnormal sperm cells are matured from damaged seminiferous tubules, for example bent mid-piece, curved mid-piece, bent tail, curved tail, normal tail without head, normal head without tail, looped tail and coiled tail. Tertiary sperm abnormalities arise from improper handling of semen samples (Adebowale *et al.* 2008).

2.24.2.4 Sperm Motility:

The assessment of sperm motility is one of the most often used parameters for semen evaluation .Sperm motility is a critical factor in the maintenance of fertility. Historically, it has been difficult to use sperm motility assessment as a predictor of fertility potential in poultry, possibly because of the subjectiveness of the methods used (King *et al.*, 2000). Spermatozoa motility is a subjective assessment of sperm cells, involving rating of semen by the character of the wave observed in fresh semen under a standard size cover slip at 37^{o}c. The character of the wave is dependent upon the activity of the spermatozoa and their concentration. Evaluation of spermatozoa motility is a good indicator of sperm viability in which case the portion of active spermatozoa in the ejaculate is estimated. Spermatozoa of low motility rating are considered an important cause of male infertility.

A more specified measure is motility grade, where the motility of sperm is divided into four different grades: (Semen Analysis Wikipedia, 2007).

Grade 4: Sperm with progressive motility. These are the strongest and swim fast in a straight line. Sometimes it is also denoted motility a.

Grade 3: (non-linear motility): These also move forward but tend to travel in a curved or crooked motion. Sometimes also denoted motility b.

Grade 2: These have non-progressive motility because they do not move forward despite the fact that they move their tails.

Grade 1: These are immotile and fail to move at all.

The motile ability of spermatozoa is believed to be initiated among others by the cyclic 3' 5' adenosine monophosphate (Cyclic AMP) and calcium ions in the caput epididymis (Olson and Danso, 1981). Motility from there on is maintained by protein in the form of acidic epididymal glycoproteins. Flickinger (1981) showed that these proteins are required for sperm maturation in the epididymis and are under the influence of androgens (Brooks and Higgins, 1980). Motility of spermatozoa is known to vary with different regions of the epididymis. They have limited capacity at the proximal end of the caput epididymis while few may start to possess capacity at the distal end, whereas, they attain 50% forward movement at the end of the segment. The attainment of full motility in about 90% of the spermatozoa has been found from the lower corpus to the upper caudal and body of the caudal epididymides (Bedford and Miller, 1978), Nwakalor *et al.* (1986) estimated a motility score of $37.1 \pm 0.12\%$ for cock semen which is low compared with 57% and 61% score reported by Abdul *et al*. (2013) and Udeh *et al.* (2001) respectively. In his report Oyeyemi *et al.* (1996) asserted that progressive motility decreased with increase in frequency of ejaculation in West Africa Dwarf buck semen. The cock spermatozoa motility is reported to be poor at the initial stage. This according to Clulow and Jones (1982) has been attributed to very little time (2hours) spent in the epididymal region, which does not allow it to undergo massive cell surface changes. Poor initial motility causes low pH which is attributed to the production of lactic acid which lowers

pH values resulting in the inactivation or loss of motility of spermatozoa, a negative correlation were established between motility and pH.

2.24.2.5 Semen Colour

The most obvious evaluation of semen quality is colour (Peter *et al*, 2008). Moss *et al*. (1979) has reported that the colour of semen is partly determined by the concentration of spermatozoa and partly by other factors like the presence of pus cells. The semen of chickens is usual white, opaque, watery or clear depending or sperm concentration (Sturkie, 1970). Boar and Stallion semen have a translucent greyish white colour (Moss *et al.*, 1979), thin and watery to a milky fluid in swamp buffalo, depending on the concentration (Jamuodeen *et al.*, 1982). The yellow turkey semen is said to contain abnormal spermatozoa, spermatids, often numerous macrophages and is of reduced fertilizing capability when compared to normal white semen (Thurston and Biellier, 1972). Semen colour has been implicated to influence fertility.

2.25 TESTICULAR MORPHOMETRY

The testes are the biological industry in male species involved in spermatogenesis and hormonal secretion. Adequate knowledge of the ability of the testes toproduce sperm cells is essential in poultry breeding (Togun and Egbunike, 2006) reported that testis size is a good indicator of the present and future sperm production in bulls. They further observed that the knowledge of basic morphometric characteristics of reproductive organs is of great value in breeding, soundness assessment and potential fertility.

Clulow and Jones 1982 reported that in the domestic fowl, it has been shown that the left gonad of the genetic male acquires many of the primordial germ cells from the right gonad early in embryonic growth (Hafez, 1993). Consequently, the left testis tends to be larger than the right. In the domestic cock, this relationship persists until about six months of age, but after that, the right testis tends to become heavier-than the left. The ratio of paired testis weight to the weight (Gonadal Index) of the Japanese quail has been reported to be 2.26±0.01, while there were no significant differences between the mean weights of the left and right testes.

CHAPTER THREE

MATERIALS AND METHODS

3.1. STUDY I: PERFORMANCE OF CHICKS FED DIETARY LEVELS OF BIOPLEX ZINC

3.1.0 INTRODUC TION

Zinc is widely distributed throughout the body and plays an essential role in many body processes. Zinc is present in many enzyme systems that are concerned with the metabolism of feed constituents. For example, zinc is a constituent of carbonic anhydrase, carboxypeptidase A and B, several dehydrogenases, alkaline phosphatase, ribonuclease and DNA polymerase. Zinc is required for normal protein synthesis and metabolism, and it is also a component of insulin so that it functions in carbohydrate metabolism. Because zinc plays so many important roles in the body, it is required by all livestock and poultry (Larry, 1987; Thomas and Ravidan, 2010).

Church *et al.* (1984) reported that dietary components have measureable effects on blood constituents; also, serum chemical values may indicate the adjustment of certain nutrient upward or downward.The blood profile has been shown to be a guide to nutritional status in feeds (Wilson and Medd, 1978). Blood parameters have been shown to be major indices of physiological, pathological and nutritional status of an organism and changes in the constituent compound of blood when compared to normal values could be used to interpret the metabolic state of animals as well as quality of the feed (Babatunde *et al.*, 1992). The blood contains a myriad of metabolites and other constituents, which provide a valuable medium for clinical investigation and assessment of nutritional status of human beings and animals. Hence, the use of blood and serum biochemical parameters in medical nutritional assessment and survey of animal (Olorode *et al.*, 1995).

The present study was designed to investigate the effect of Bioplex zinc on the growth, haematology and serum biochemical indices of chicks.

3.1.0.1 Experimental Site

The feeding trial was carried out in the Poultry Unit of the Teaching and Research Farm, University of Ibadan, Ibadan, between December 2010 and July 2011, and further laboratory analyses were carried out at the Animal Physiology Laboratory of the Animal Science Department and Veterinary Pathology Department, both of the University of Ibadan, Ibadan.

3.1.0.2 Experimental Birds

A total of 240 day-old Nera black cockerels used for this study were purchased from Ajanla farm in Ibadan, Oyo State, Nigeria.

3.1.0.3 Experimental Diets

Four experimental diets were formulated. Control (diet 1) had non-inclusion of Bioplex zinc, Diets 2, 3 and 4 had 100mg/kg, 200mg/kg and 300mg/kg inclusion of Bioplex zinc respectively. The Bioplex zinc was added on top of the feed. The diets were isocaloric and isonitrogeneous and satisfied the nutrient requirements of the birds at the various physiological phases as recommended by National Research Council (1998). The gross and proximate composition of the chicks' starter diets are shown on Tables 1 and 2 respectively.

3.1.0.4 Animal Management Practices

Before the arrival of the day old chicks, the brooder house, feeder trays and drinkers were thoroughly washed and disinfected. Black polythene sheets were used to cover the sides of the brooder house to conserve heat. Heat and light sources such as electricity bulbs and charcoal pots were adequately provided. On arrival, an antistress (commercial vitalite) was administered to the chicks; they were raised in the brooding unit for two (2) weeks during which they were fed the control diet.

3.1.0.5 Experimental Layout and Feeding Trial

After two weeks of physiological stabilization period, the chicks having initial average weights of 99.75g were randomly allotted to four experimental diets in a completely randomized design (CRD). Each experimental diet had 60 birds replicated five times with 12 birds per replicate. The feeding trials for the starter phase birds were for eight weeks. The birds were provided fresh, clean and appropriate feed *ad libitum* daily throughout the feeding period. The birds were also given intra-ocular vaccination at day old against New castle disease while other routine vaccination, medication and good hygienic conditions were strictly followed. The weekly feed intake and body weight gains were monitored, and blood samples collected for haematology and serum biochemical indices.

Table 1: Gross composition (%) of experimental diet fed to chicks 2-8 week (starter phase)

Feed Ingredients	**(%)**
Maize	57.25
Soyabean meal	25.30
Wheat offal	4.00
Groundnut cake	8.43
Fish meal	2.50
Dicalcium phosphate	1.50
Methionine	0.22
Common Salt	0.30
Vitamin-Mineral Premix	0.50
Total	**100.00**
Calculated Nutrients	
Crude protein (%)	22.24
Crude fibre (%)	3.50
Metabolisable energy (kcal/kg)	2797.00
Zinc in the diet (mg/kg)	58.99

Vit+mineral mixture provides per kg of diet. Vitamin A- (50,000,000 i.u) ; Vitamin D_3-(1,600,000i.u) ; Vitamin E(20,000mg) ; Thiamin (2,000 mg) ; Riboflavin (5,000 mg) ; (D-pantothenic acid 10,000 mg) ; Vitamin B6 (3,000 mg) ; Vitamin B_{12} (20 mg) ; Vitamin K (1,000 mg) ; Vitamin C (100,000 mg) ; Nicotinic acid (100,000 mg) ; Folic acid (600mg) ; Biotin (0.5 mg) ; Zinc (40,000 mg) ;Copper (5,000 mg) ; Iodine (200mg) ; Cobalt (250 mg) ; Selenate (125mg) ; Zinc bacitracin (15,000mg) ; Farmers UGf 75,000 mg, Choline chloride (400,000 mg).

3.1.0.6 Data Collection

3.1.0.6.1 Feed Intake

Feed consumption by the birds was determined weekly as the difference between the weekly feed supplied and left over.

3.1.0.6.2 Weight gain

The live weight changes of the chicks were taken weekly throughout experimental periods. The average weight gain of chicks per week was obtained by taking the differences between mean weights for two successive weeks. From the average weekly weight gain, the average daily weight gain of the chicks was estimated.

3.1.0.6.3 Feed Conversion Ratio (FCR)

The feed conversion ratio was calculated using the formula stated below:

$$\text{Feed conversion ratio} = \frac{\text{Feed intake}}{\text{Weight gain}}$$

3.1.0.6.4 Haematological Parameters

At the end of the feeding trial of eight weeks, 2 chicks per replicate were starved overnight before blood collection. The birds were bled by jugular veins puncture and blood samples collected using vacutainer–glass tubes containing anticoagulant (ethylene diamine tetra acetic acid EDTA) for determination of haematological parameters.

3.1.0.6.4.1 Red blood cell (RBC) determination

The red blood cell was determined using improved Neubauer haemocytometer after the appropriate dilution at a ratio of 1:200 (blood: red blood cell diluting fluid) and then calculated with the formula below:

RBC/ul= number of red blood cells counted x 5 x 10 x 200.

3.1.0.6.4.2 Haemoglobin (Hb) determination

The Hb was determined by cyanmethaemoglobin method as described by Coles (1986) 0.02ml of blood was expelled unto 4ml drubkins solution. The mixture was allowed to stand for 5 minutes for full colour development.

Sample haemoglobin concentration was obtained using this relationship:

Sample haemoglobin= Reading of test X standard haemoglobin concentration (g/100ml)

Reading of standard

3.1.0.6.4.3 Packed Cell Volume (PCV) determination

This was determined by spinning about 75 μL of each blood sample in heparinized capillary tube in a haematocrit centrifuge for about 5 minutes. PCV was read on Wintrobe microhaematocrit reader as described by Benson *et al.* (1989)

3.1.0.6.4.4 Measurement of blood indices and corpuscular constants

The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined using the appropriate formulae described by Jain (1986)

$$\text{MCV } (\mu^3) = \frac{PCV}{RBC} X 10$$

$$\text{MCH } (\mu\mu g) = \frac{HB}{RBC} X\ 10$$

$$\text{MCHC } (\%) = \frac{Hb}{PCV} X\ 100$$

3.1.0.6.4.5 White Blood Cell (WBC) and Differential Leukocyte counts.

The total leukocyte counts was determined using improved Neubauer haemacytometer after appropriate dilution at a ratio of 1:20 (blood: white cell diluting fluid) and differential leukocyte counts performed using the oil-immersion objectives examination of blood films stained with Modified Romanovsky's Giemsa Stain for 30 minutes, rinsed in water and air-dried.

Samples of WBC counts were obtained using the relationship:

WBC/ μl= Numbers of white blood cells counted x 0.25 x 10 x 20.

3.1.0.6.5 SERUM BIOCHEMICAL PARAMETERS

The procedure of blood collection was as described in section 3.2.6.4; however the blood samples for biochemical studies were collected in vacutainer tubes without anticoagulant. The tubes were kept in a slanting wooden rack, and the blood allowed to clot. The serum was separated clearly by decanting after the blood samples were spin in a centrifuge at 3000 rpm for 10 minutes. The samples were kept in sterile vacutainer tubes and kept deep frozen prior to analysis.

3.1.0.6.5.1 Determination of Serum Total Protein and Albumin

The serum total protein of the chicks was determined by the Biuret method described by Kohn and Allen (1995) while albumin value determined using the Bromocresol Green (BCG) method as described by Peters *et al.* (1982)

3.1.0.6.5.2 Determination of Serum Globulin and Albumin/Globulin ratio

The serum globulin concentration was obtained by subtracting albumin from the total protein while the albumin/globulin ratio was obtained by dividing the albumin value by the calculated globulin value.

3.1.0.6.5.3 Serum Enzymology

Serum enzymes, Aspartate amino transferase (AST), Alanine aminotransferase (ALT), and Alkaline Phosphatase (ALP) activities were determined using the spectrophotometric methods of Hoder and Rej (1983) using the appropriate reagent kits by Randox Laboratories Limited, United Kingdom.

3.1.0.6.5.4 Other Serum Analytes

Glucose, Creatinine, Urea, Cholesterol and Triglycerides were determined following the procedures described by Davice and Lewis (1991).

3.1.0.6.6 Proximate Analysis

The proximate analyses of the experimental diets of the chicks were carried out according to the procedure described by Association of Analytical Chemists (AOAC, 1999)

3.1.0.6.7 Statistical Analysis

The design used for the experiment was one – way ANOVA. All the data obtained were subjected to Statistical Analysis of Variance (ANOVA) Procedure of SAS, 2001 package. The significant treatment means were compared using the Duncan option of the same software.

3.2: STUDY 2: GROWTH, HAEMATOLOGICAL, SERUM PARAMETERS, CARCASS AND RELATIVE ORGAN CHARACTERISTICS OF GROWING COCKERELS FED DIETARY LEVELS OF BIOPLEX ZINC

3.2.0 INTRODUCTION

Blood contains a myriad of metabolites and other constituents which provide a valuable medium for clinical investigation and assessment of nutritional status of human being and animals (Wilson and Medd, 1978). The analysis of blood has become important in assessing the physiological and pathological changes occurring in animals and it is used in assessing the body's ability to respond to external invasion of the body. Breazile (1971) has reported negative effects of the use of unusual ingredients on some blood parameters as a result of factors such as nutrients imbalance, improper metabolism, presence of antinutritional factors and toxic elements in the ingredients. Serum biochemical values may indicate the adjustment of certain nutrient upward and downward. Also an estimation of the total quantity of serum proteins may be utilized as an indication of the nutritive state of the animal.

Carcass composition studies are important aspect of science of meat production fron animals. Carcass quality is a measure of carcass palatability and acceptability. There is need for a continued research on performance of different breeds of livestock on different management systems and their nutritionat requirement if intelligent breeding and management plans are to be formulated for their improvement.Variations in carcass qualities are mainly due to breed, sex, age and nutritional status of the animal. There is a challenge to develop data based on a system that captures information on performance of livestock on different feeding regimes and effect on carcass qualities (Alaku, 1997).

The objective of this study was to determine the effect of dietary Bioplex zinc on growth, haematology, serum biochemistry, carcass and organs' characteristics of the blood of growing cockerels.

3.2.0.1 Experimental materials and operations

The same sets of experimental birds used in sytudy 1 were used in a 10-week feeding trial for the grower phase. Details of the experimental site, experimental birds, experimental diets, experimental design, management practices and experimental layout and feeding trial are as described in the starter phase of this thesis. The Gross composition (%) of growing cockerels fed dietary levels of Bioplex zinc is as shown in Table 6.

3.2.0.2 Haematological parameters

At the 16th week of the feeding trial, 2 birds per replicate were starved overnight before blood collection. The birds were bled by jugular veins puncture and blood samples collected using vacutainer–glass tubes containing anticoagulant (ethylene diamine tetra acetic acid EDTA) were used for the determination of red blood cell, haemoglobin, packed cell volume, white blood cell and differential leukocyte counts using their standard procedures as recorded in the starter phase.

3.2.0.3 Serum biochemical parameters

The procedure of blood collection was as described in study 1. The serum total protein, albumin, globulin, albumin/globulin ratio, glucose, urea, creatinine, cholesterol, triglycerides, alanine amino transferase, aspartate amino transferase, alkaline phosphates were also determined.

3.2.0.4 Carcass evaluation

Two birds were slaughtered from each treatment. The live, bled, and plucked weights were determined using a top loading digital scale. The weights of the drumstick, wings, shank, breast, back, head, neck and thigh were determined using an electronic scale. Thereafter, the relative weights of the internal organs (liver, kidney, heart, lungs, spleen,

gastro intestinal tract GIT, gizzard, crop and bile) were also determined using the same weighing balance.

3.2.0. 5 Organoleptic characteristics

This was carried out using ten-trained panelist drawn from the students of the Department of Animal Science, University of Ibadan, Ibadan. Samples for sensory evaluation were obtained from each of three cuts (Breast, Back and Drumstick) and cooked in boiling water for 20 minutes using electric stove. These samples were wrapped in imperious polythene pouches, which could not be destroyed by cooking process. No spices were added to these. The assessment was based on a nine-point hedonic scale. The score was arranged in a descending order, the maximum score of 9 being given to extremely liked condition while the lowest score 1 was for the poorest condition (Mahendrakar *et al.*, 1981)

3.2.0.6 Data Collection

3.2.0.6.1 Performance characteristics

At the beginning of each week, weighed quantities of the experimental diets were put in individual plastic containers with covers and each bearing the treatment of the birds. Each bird was supplied feed from the plastic containers throughout the days of the week. At the end of the weeks, the remnant in the plastic container and feeder was weighed. The difference between the quantity supplied and the remnant gave the feed intake per week per bird from which the average daily feed intakes were estimated for each bird. The live weight changes of the chicks were taken weekly throughout experimental periods. The average weight gain of chicks per week was obtained by taking the differences between mean weights for two successive weeks. From the average weekly weight gain, the average daily weight gain of the chicks was estimated. The feed conversion ratio was calculated using the formula stated in study 1.

3.2.0.6.2 Carcass characteristics of organs

At the end of the feeding trial, two birds per replicate were randomly selected for organ analysis. Selected birds were starved of feed and water overnight and their live weights were taken. They were then slaughtered and properly bled before scalding in hot water at 65^{o}C. Feathers were removed after scalding and the plucked weight obtained was expressed as percentage of live weight. The eviscerated weights were also recorded and expressed as percentage of live weights. The liver, kidney, spleen, heart, lung, pancreas, testes and Gastro Intestinal Tract (GIT) were weighed and expressed as percentage of live weights as follows.

$$\text{Relative organs weight} = \frac{\text{Organ weight}}{\text{Live weight}} \times 100$$

3.2.0.7 Proximate Analysis

The proximate analyses of the experimental diets of the growing cockerels were carried out according to the procedures described by AOAC (1999)

3.2.0.8 Statistical Analysis

The design used for the experiment was Complete Randomized Design (CRD). The data obtained were subjected to analysis of variance (ANOVA) procedure of statistical analysis software (SAS, 2001) using initial body weights as covariant. The treatment means were compared using the Duncan's procedure of the same software.

3.3. STUDY 3: PERFORMANCE, NUTRIENT DIGESTIBILITY, ZINC RETENTION AND HISTOPATHOLOGICAL EXAMINATION OF COCKS FED DIETARY LEVELS OF BIOPLEX ZINC

3.3.0 INTRODUCTION

The quality of feed materials is determined by the amount of nutrients contained within the material, and how efficiently it can be digested and released for absorption and utilisation within the body. It is important that the balance of nutrients is correct, as otherwise deficiencies or toxic levels can cause disease or incorrect growth. Likewise it

is essential that nutrients are available in correct forms so that the body is able to breakdown and use them more efficiently and effectively (Leeson, 2005).

Zinc is a unique mineral element which is essential for peak production of poultry for adequate immune function, enzymatic function and blood synthesis in rapidly growing broilers and turkeys. Integrity of skin and feet are also vital to bird health and profitability. Intensive modern production environments often place birds under stress, increasing the chance of infection or disease. Healthier birds create greater value for both the processor and producer (Leeson, 2005).

Effective mineral nutrition means supplying birds' mineral requirements in sufficient quantities and forms to maintain bird health and to meet the demands of modern production. Research has shown that minerals in the form of oxides or sulphates often provide only short-term benefits. A primary reason for their inefficiency is their frequent tendency to interact with other minerals in the diet. Transition trace elements such as iron, zinc, copper and manganese are weakly charged and, therefore, easily bound to other minerals, reducing their potency. A more effective method is to chelate the minerals, by attaching them to a series of amino acids, di-peptides and tri-peptides for the journey through the layers of the digestive tract and into the cells where they are absorbed and utilised.(Ao *et al*., 2012).

Poultry derives the minerals required for normal growth and metabolism from the diet. The biological availability of a mineral from the diet is manifested by the efficiency with which the body utilises and retains the dietary mineral. The retention will be influenced by a number of dietary factors, including diet or ingredient type, source of minerals and, levels and relative proportions of various minerals. Published data on the effects of diet type, however, are limited (Thomas and Ravindran, 2010). In the 1990's, a greater availability for some organic trace mineral sources than for the inorganic forms was reported, leading to an increased interest in the feed industry for these products. Trace minerals from organic sources would appear to be protected from forming insoluble complexes with feed or endogenous components present in the digestive tract. Moreover

trace mineral complexing or chelating to small size organic molecules would enhance their absorption and even improve their metabolic utilization (Nollet *et al*., 2007).

This work was therefore undertaken to study the effect of dietary levels of Bioplex zinc on nutrient digestibility, performance, zinc retention and histopathological examination of cocks.

3.3.0.1 Experimental materials and managment

The same sets of experimental birds used in study 2 were used in a 12-week feeding trial for the finisher phase. Details of the experimental site, experimental birds, experimental diets, experimental design, management practices and experimental layout and feeding trial are as described in the growers' phase of this thesis. The gross composition of cocks fed dietary levels of Bioplex zinc is shown in Table 6.

3.3.0.2 Organs histopathological examination

This was carried out in the Department of Pathology, Faculty of Veterinary Medicine, and University of Ibadan. Two (2) birds per replicate were sacrificed and used for histological analyses. The spleen, liver, kidney and testes were carefully removed from each of the birds and weighed using an electrochemical balance and thereafter subjected to each of the following procedures:

3.3.0.3 Fixation:

Samples from the spleen, liver and kidney were fixed in 10% formalin while the testes were fixed in Bouin's fixative for 24 hours.

3.3.0.3.1 Dehydration and Embedding:

Following fixation, the specific sections of the organs collected were left in (70%, 80%, 90%, 95%, 95% and 100%) of one hour interval each of different ethanol concentration and thereafter cleared in two changes of xylene. The organs were allowed one hour to impregnate in motten paraffin wax at 60^{o}C. Histological sections for 4 microns thick were floated and flattened out on 40^{o}C water and then picked up carefully with clean slides smeared with Mayer's egg albumin. The slides were stored in an incubator for 30 minutes before staining with haematoxylin-eosin (H&E), covered with coverslips and

DPX mountant and examined under the microscope for pathological examinations as described by Rowet, 1962.

3.3.0.4 Nutrient Digestibility

At the 18th and 24th weeks of the feeding trial, 5 birds per treatment were housed in specially adapted metabolic cages. Feed intake and faecal output were weighed daily. Faecal collection was done for a period of five days. Droppings from each bird were collected weighed, mixed and aliquots taken daily. The daily aliquots and the respective feed samples for the birds were oven-dried at 105°C per 24 hours to determine (their moisture content) for future analyses.

$$\text{Dry matter digestibility (DMD)} = \frac{\text{Feed intake (gm) DM} - \text{Faecal output (gm) DM}}{\text{Feed intake (gm) DM}}$$

Zinc Retention was calculated by the formulae:

$$= \frac{\text{Zn absorbed (g/day)} \times 100}{\text{Zn intake (g/day)}}$$

3.3.0.5. Chemical Analyses.

The method of A.O.A.C (1999) was used to determine the chemical composition of the experimental diets and faecal samples collected. The feed and faecal samples collected were analyzed for zinc using Atomic Absorption spectrophotometer as described by Johnson (1982). For the analytical procedures, the oven-dried samples were ground. 2g of each were weighed and digested in a lightly covered conical flask, using 25ml of HNO_3, and 25ml of H_20_2 at very low heat until the flask content was reduced to one-third its original volume. The digesta were filtered and made up to 100ml with deionized water in a standard volumetric flask. Contents of Zn in each of the samples were aspirated and determined using appropriate lamp into an atomic absorption spectrophotometer.

3.3.0.6 Statistical Analysis

The design used for the experiment was Complete Randomized Design (CRD). The data obtained were subjected to analysis of variance (ANOVA) procedure of statistical analysis software (SAS, 2001) using initial body weights as covariant. The treatment means were compared using the Duncan's procedure of the same software.

Linear and polynomial regressions analyses were carried out to investigate relationships among the daily feed intake, weight gain and BZ inclusion levels respectively. These generated the coefficient of determination (R^2) to indicate efficiencies of prediction.

The regression model was used Y = a+bx

Where Y= dependent variable

a = intercept

b = regression coefficient of parameters determined

x = independent variable i.e. the BZ inclusion levels

3.4 STUDY 4: SEMEN CHARACTERISTICS OF COCKS FED DIETARY LEVELS OF BIOPLEX ZINC

3.4.0 INTRODUCTION

Zinc participates as a co-factor or component of more than 240 enzymes, being important for protein and carbohydrate metabolism, growth, and reproduction (Steveton, 2009). Zinc (Zn) is an essential mineral for animal development and function. Trace minerals are important nutrients in diets for poultry. They are required for growth, bone development, feathering, enzyme structure and function, and appetite. They predominantly act as catalysts in many enzyme and hormone systems (Underwood and Suttle, 1999). The reproductive potential of poultry birds (cocks) is determined to large extent by the quality of the semen it produces. The importance of semen evaluation in poultry breeding (Natural and artificial breeding) for selecting breeding males or for routine monitoring of their reproductive performance cannot be overemphasized Ajayi *et al*. (2011). Zinc is important for the cell division and the production of healthy sperm. It is the most critical trace mineral for male sexual function. It is needed for

testosterone and hormone metabolism, testicle growth, sperm production, motility, count, reducing excess estrogen in male reproductive tissue. Zinc is needed for progesterone synthesis and a deficiency can produce excessive prolactin secretion. Zinc helps in protecting the structure of the genetic material or the DNA chromatin in the sperm nucleus.This structure is important for successful fertilization (David and Watts, 1988).

Normally, zinc concentrations are very high in the male genital organs, particularly in the prostate gland, which is largely responsible for the high zinc content in seminal plasma. Spermatozoa themselves also contain zinc, which is derived from the testis (Niola and Salis, 2011). Deficiency symptoms include disturbances of many metabolic processes, resulting in lower production performance, loss of appetite, reproductive disorders or impaired immune response (Vander Klis and Kemme, 2002). In a reviewed article of the role of minerals in fertility and reproductive diseases of dairy cattle Wilde (2006) highlighted that organic forms of Zn are better retained than inorganic sources and so may provide greater benefit in disease prevention, notably mastitis and lameness. Although, there are research findings on semen evaluative parameters in cocks (Ezekwe *et al*. 2003; Oguike *et al*. 2000; Egbunike and Oluyemi, 1979). There is little or no information on the influence of Bioplex Zinc on the semen characteristics (quantity and quality) of cocks. There is also paucity of information in the available literature on the morphometric characteristics of reproductive organs and predictive reproductive efficiency in poultry breeds of chickens (Obidi *et al.*, 2008).

Based on the aforementioned situation, the present study was designed to investigate the effect of Bioplex zinc on the reproductive performance of adult cocks.

3.4.1 Experimental materials and management

The same sets of experimental birds in study 3 on a 12-week feeding trial for the finisher phase were used. Details of the experimental site, experimental animals, experimental diets, preexperimental operations, experimental layout and feeding trial are as described in study 3 of this thesis. The gross composition of cocks fed dietary levels of Bioplex zinc is shown in Table 6

3.4.2 Age at attainment of puberty

Two cocks per replicate from each treatment were subjected to the double hand lumbar massage system of Burrows and Quinn (1937) at 18 weeks of age to induce ejaculation. This exercise was repeated thrice a week to establish the exact period puberty was attained by the birds. Age at puberty was taken as the period when spermatozoa were observed in the cloacal fluid of at least 50% of cocks on each treatment.

3.4.3 Collection of semen

After the cocks that were subjected to the prior training were confirmed to have attained puberty and became used to the system of semen collection, they were ejaculated at 72 hours intervals at 22 weeks of age. A total of five semen collections were made for each bird. A calibrated micro syringe was used to collect exudates from the base of the papillae of the birds. The exudates was smeared onto a glass slide and later examined under microscope for the presence of spermatozoa which is taken as an index of puberty

3.4.4 Semen evaluation and analysis

3.4.4.1 Volume of ejaculate

The semen volume was read directly on the calibrated micro syringe to the nearest 0.01ml and recorded.

3.4.4.2 Semen Colour

This was visually appraised directly from the calibrated measuring micro syringe under natural light. The rating was from creamy white to translucent.

3.4.4.3 Mass Activity

A drop of undiluted semen was placed on a clean glass slide and examined with microscope under low power. The mass activity of spermatozoa for each bird was scored according to the intensity of the wave motion from absence of wave motion (0), to slow swirling (+), rapid swirling (++) and turbulent swirling (+++) characterized by the appearance of dark prominent waves in a very rapid motion.

3.4.4.4 Sperm Motility

Sperm progressive motility was determined immediately after collection. A drop of undiluted semen was mixed with a drop of warm physiological saline on a clean glass slide, and a cover slip was applied. The percentage of progressive motile spermatozoa was estimated by rapid observation of 8 to 10 fields under low power microscope and the motility score rated between 0-100 for each sample.

3.4.4.5 Sperm Concentration

The concentration of spermatozoa in the ejaculate of individual animals was estimated by haemacytometric counts based on the same principles as in red blood cell count. One part of thoroughly mixed semen diluted in 1:99 parts of formal saline (90% physiological saline+10% formalin) in a blood pipette. Improved neubauer haemocytometer chamber was then charged by allowing the mixture to run, by capillary action, under the cover slip of the haemocytometer. Within the smaller squares of the chamber, sperm cell counting was taken in the five squares diagonally from the upper left to the lower right of the haemocytometer. Only those sperm cells that are entirely inside the centre of the squares were counted. Also, only head regions of the sperm cells on the top and those in the left boundary lines were counted. The total number of sperm cells counted in the square was multiplied by a dilution factor of 200 and by a constant 10 thousand (5 x 200 x 10,000) or 0.01 x 10^9 to give the sperm concentration per ml of semen (Polakoshi and Zaneveld, 1977).

3.4.4.6 Live-Dead Ratio of Sperm Cells

The proportion of live/dead spermatozoa was determined by adding a drop of the staining solution (Eosin-Nigrosin) to a drop of undiluted semen on a clean glass slide. They were mixed gently and a smear was made by drawing a glass slide over the slide containing the semen and eosin-nigrosin mixture. The slide was then air-dried and covered with a cover slip and observed under oil immersion. Unstained spermatozoa were alive when staining was applied; whereas the stained spermatozoa were dead and appeared darker.

3.4.4.7 Sperm Morphology

Sperm morphology is one piece of the semen analysis puzzle. Sperm morphology refers to the size and shape of the sperm. When sperm morphology is tested, they look for a percentage of sperm that seem to appear normal when viewed under a microscope. Abnormal sperm morphology is one cause of male infertility (Semen Analysis Wikipedia, 2007).

The proportion of abnormal spermatozoa was evaluated under the microscope at a 10 x 10 magnification by random evaluation of at least 100 spermatozoa on the slide prepared from the live-to-dead sperm estimation. Primary sperm abnormalities observed and recorded include. head, midpiece and tail defects. These abnormal sperms have very difficult time reaching the egg and fertilizing it.

3.4.4.8 Testes Volumes

Testes volume was determined individually by filling a 25mls-measuring cylinder with water to a known volume after which the testis was carefully dropped into the cylinder, the amount of water displaced was recorded as the volume of the testis. The testis density was calculated from the weight and volume of the testis and expressed in gram/cubic centimeter.

3.4.4.9 Paired Testes weight

The right and left testes of each cock sacrificed from each treatment was carefully removed and measured separately. Their weights were recorded to the nearest 0.01gram. The right and left epididymidal weights were also measured separately to the nearest 0.01gram.

3.4.5. Testicular Histology

3.4.5.1 Fixation

Samples from the right testis of cocks from each treatment were fixed in aqueous Bouin's fixative for 12 hours. Bouin's fixative is composed of 75ml of picnic acid, 25ml of formalin and 5mls of glacial acetic acid. Following fixation, the tissue samples were subjected to further histological processes as described by Rowett (1962).

3.5.5.2 Histological slides preparation

Samples of testes fixed in Bouin's fixative were taken from the equatorial regions of the testes washed in (70%, 80%, 90%, 95%, 95% and 100%) ethanol for one hour each and cleared in xylene before being embedded in motten paraffin wax (Magumdar, 1980). After embedding, tissue samples were cut (sectioned) at 4 microns using a microtome (Rotary Kepee model KD202A). Staining was done with haematoxylin-eosin and slides were prepared from the tissues. The slides were read from histological indicators in order to observe possible degenerative changes on the organs testicular structures using a microscope connected to a computer system. A photomicrographic software Phoenix Micro Image Analysis (2002) version 1.33 was used to project the slides on computer for clear assessment. The slides were subsequently captured and printed for interpretation

3.4.6 Statistical Analysis

The design used for the experiment was Complete Randomized Design (CRD). The data obtained were subjected to one-factor analyses of variance using the analysis of variance (ANOVA) procedure of statistical analysis software (SAS, 1999). The treatment means were compared using the Duncan's procedure of the same software.

CHAPTER FOUR

RESULTS

4.1. STUDY I: PERFORMANCE OF CHICKS FED DIETARY LEVELS OF BIOPLEX ZINC

4.1.1 Proximate composition of experimental starter diets

The proximate composition of the chicks' starter diets is shown in Table 2. The feed met with the nutritional requirement of cockerel chicks and is in line with the standards of National Research Council (1994). The diets crude protein varied between 21.96% and 22.30% with birds on treatment 4 having the seemingly highest value of 22.30% and treatment 3 with the lowest value of 21.96%. The crude fibre ranged from 3.48% in treatment 4 to 3.50% in treatment 1 (control) and treatment 2 respectively. The ether extract content varied from 2.15% in treatment 1 (control) to 2.70% in treatment 3. The ash content of the experimental diets varied from 12.00% in treatment 1 to 12.25% in treatment 4 (control). The Nitrogen free extract content ranged between 59.69% and 60.31% with treatment 1 (control) having the highest value and treatment 3 having the least value.

Table 2: Proximate composition (g/100g DM) of experimental diets fed to chicks (2-8 weeks)

Nutrients	Dietary Zinc Levels (mg/kg)				
	T1	T2	T3	T4	SEM
	O (Control)	100	200	300	
Moisture	10.40	10.50	10.45	10.60	0.03
Dry Matter	89.60	89.50	89.56	89.40	0.34
Crude protein	22.04	22.13	21.96	22.30	0.40
Crude Fibre	3.50	3.50	3.49	3.48	0.03
Ether Extract	2.15	2.20	2.70	2.40	0.02
Ash	12.00	12.10	12.16	12.25	0.03
Nitrogen Free Extract	60.31	60.07	59.69	59.57	0.15

4.1.2 PERFORMANCE CHARACTERISTICS OF EXPERIMENTAL CHICKS

The performance data of the chicks fed dietary levels of Bioplex zinc is as shown in Table 3. The daily weight gain and final body weight of the birds fed dietary Bioplex zinc were significantly ($p<0.05$) different from birds on treatment 1 (control).

The daily feed intake of the chicks apparently increased as the dietary Bioplex zinc levels increased. The daily weight gain followed the same trend as the daily feed intake with treatment 1 (control) having the least value of 6.23% and treatment 4 having the highest value of 6.95%. The daily weight gain of birds fed diets 2, 3 and 4 were not significantly dfferent from one another but they were significantly ($p<0.05$) higher than those fed control diet (1). The final body weight of birds fed diet 1 was significantly ($p<0.05$) lower than those fed diets 2, 3 and 4. However, final body weight of birds fed diets 2 and 3 were not significantly different from those fed diet 4. The feed conversion ratio of the birds decreased with increase in the dietary Bioplex zinc levels. The highest value was obtained for chicks fed treatment 1 (control) (4.94) while the least was observed for chicks on treatment 4 (4.60) although there were no statistical differences across the treatments.

Table 3: Performance characteristics of chicks fed dietary levels of Bioplex zinc

Parameters	Dietary Zinc Levels (mg/kg)				
	T1 O (Control)	**T2 100**	**T3 200**	**T4 300**	**SEM**
Initial body weight (g/bird)	100	100	99	100	
Daily feed intake(g/bird)	30.78	31.11	31.53	31.93	0.27
Daily weight gain (g/bird)	6.23[b]	6.61[a]	6.78[a]	6.95[a]	0.06
Final body weight (g/bird)	361.29[b]	377.72[a]	383.94[a]	391.82[a]	2.33
Feed conversion ratio	4.94	4.70	4.65	4.60	0.04

a,b,c: Means along the same row with different superscripts are significantly different ($P<0.05$)

SEM: Standard Error of Means

4.1.3 HAEMATOLOGICAL PARAMETERS

The haematological values of chicks fed different levels of dietary Bioplex zinc are presented in Table 4. All the parameters measured: Packed cell volume, Haemoglobin, Erythrocytes, MCV, MCH and MCHC were not significantly different among the dietary treatments except the white blood cells and some of the differential counts.

The mean values of the packed cell volume for the experimental chicks were not significantly different as the dietary Bioplex zinc level increased. The packed cell volume value for chicks fed treatment 2 and 4 were the same (24.60%), but were smaller than those fed treatment 3 (25.00%). The dietary levels of Bioplex zinc had no significant effects on the haemoglobin concentration of the experimental chicks. The apparently highest value of 8.38g/dl was observed for chicks fed on treatment 3 while the least value of 8.00g/dl was observed for those fed treatment 1 (control).

The erythrocytes of experimental chicks fed all the treatments were not significantly influenced by the dietary levels of Bioplex zinc. Chicks fed treatment 3 had the highest value of 2.59 ($10^6/mm^3$) while the least value of 2.38 ($10^6/mm^3$) was observed for those fed treatment 4. The mean cell volume MCV, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were not significantly ($P>0.05$) altered by the dietary levels of Bioplex zinc. The values for the corpuscular constants did not follow a particular trend.

The white blood cell values for the experimental chicks fed treatments 2 (16.88 x $10^3/mm^3$), 3 (18.00 x $10^3/mm^3$) and 4 (16.59 x $10^3/mm^3$) were not significantly different and were significantly lower ($P<0.05$) than those birds fed treatment 1 (control) (22.21 x $10^3/mm^3$). As the dietary level of Bioplex zinc increased the heterophils of the experimental chicks were significantly lowered. Chicks on treatments 3 (30.40%) and 4 (30.50%) were not significantly different from chicks on treatments 1 (34.25%) and 2 (28.60%) while chicks on treatments 1 and 2 were significantly different ($P<0.05$) from each other. The lymphocyte values for the experimental chicks fed on treatment 2 (64.80%), treatment 3 (63.20%) and treatment 4 (63.70%) were not significantly different but were significantly higher ($P<0.05$) than those chicks fed treatment

1 (56.15%). The Heterophils: Lymphocytes (H: L) ratio values were significantly different across the treatments. Birds on treatments 2 (0.44), 3 (0.48) and 4 (0.49) were not significantly different but had lower significant values than birds fed treatment 1 (0.61).

The mean values obtained for monocytes of chicks fed dietary levels of Bioplex zinc were significantly ($P<0.05$) influenced. Chicks on treatments 1 (5.40%) were significantly ($P<0.05$) different from chicks on treatment 3 (2.60%) and treatment 4 (2.00%), however chicks on treatments 2, 3 and 4 were not significantly different. The values obtained for eosinophils from the chicks fed dietary levels of Bioplex zinc were significantly ($P<0.05$) different, chicks on treatments 3 (3.70%) and 4 (3.60%) were significantly similar but different from chicks on treatments 1 (4.20%) and 2 (2.40%). The dietary Bioplex zinc had no significant influence on the basophils of the experimental chicks as the mean values obtained for each of them were not significantly different among the treatments.

Table 4: Haematological responses of chicks fed dietary levels of Bioplex zinc at the starter phase (2-8 weeks)

	Dietary Treatments (mg/kg)

Parameters	T1	T2	T3	T4	SEM
	0 (Control)	100	200	300	
Packed cell volume (%)	24.00	24.60	25.00	24.60	1.33
Haemoglobin (g/dl)	8.00	8.06	8.38	8.20	0.12
Erythrocytes (RBC) (10^6/mm^3)	2.40	2.42	2.59	2.38	0.53
MCV (μ^3)	102.42	105.90	100.55	100.45	1.58
MCH (uug)	32.43	31.38	32.46	34.14	0.82
MCHC (%)	33.30	33.30	33.30	33.30	0.00
White blood cell (10^3/mm^3)	22.21^a	16.88^b	18.00^b	16.59^b	0.48
Heterocytes (%)	34.25^a	28.60^b	30.40^{ab}	30.50^{ab}	0.62
Lymphocytes (%)	56.15^b	64.80^a	63.20^a	62.70^a	1.86
Heterophils: Lymphocytes ratio	0.61^a	0.44^b	0.48^b	0.49^b	0.01
Monocytes (%)	5.40^a	4.20^{ab}	2.60^b	2.00^b	0.34
Eosinophils (%)	4.20^a	2.40^c	3.70^b	3.60^b	0.44
Basophils (%)	0.00	0.00	0.10	0.20	0.00

a,b,c: Means differently superscripted across the rows are significantly different (P<0.05)
SEM: Standard Error of Means
MCV: Mean Corpuscular Volume
MCH: Mean Corpuscular Haemoglobin
MCHC: Mean Corpuscular Haemoglobin Concentration
*Mitruka and Rawnsley 1981

4.1.4 SERUM BIOCHEMICAL PARAMETERS

Data on serum protein quality, enzyme activities and some chemical components in the serum of chicks fed dietary levels of Bioplex zinc are presented in Table 5.

The mean values of serum total protein, Albumin, Globulin, Albumin/globulin ratio of chicks fed dietary levels of Bioplex zinc were not significantly different from one another among the treatments.

The glucose value obtained for chicks fed treatment 1 (146.15 mg/dl) were significantly ($P<0.05$) higher than chicks on treatments 2 (143.07mg/dl), 3 (141.45mg/dl) and 4 (140.20 mg/dl) which were not significantly different. The dietary level of Bioplex zinc had no significant influence on the urea and creatinine of the experimental chicks as the mean values obtained for each of them were not significantly ($P>0.05$) different among the treatments. The mean values were not also consistent in trend for the two parameters.

The mean values of cholesterol for experimental chicks fed treatments 2 (136.35mg/dl), 3 (138.50mg/dl) and 4 (137.58 mg/dl) were not significantly different from one another but were all significantly ($P<0.05$) lower than chicks on treatment 1 (control) (149.23mg/dl). As the dietary levels of Bioplex zinc increased the triglyceride mean values of the birds decreased. Chicks on treatment 1 (110.38mg/dl) had a higher significantly ($p< 0.05$) triglyceride value than chicks on treatments 2 (102.93mg/dl), 3 (100.45mg/dl) and 4 (101.84mg/dl) which were all significantly similar. The values obtained for serum Aspartate amino transferase AST showed that significant differences existed among the treatment means. The means for chicks on treatments 1 (111.89 i.u/L), 2 (106.08 i.u/L) and 3 (105.15 i.u/L) were not significantly different from one another but they were significantly higher than those on treatment 4 (100.50 i.u/L). Birds on treatment 1 were significantly different from birds on treatment 4. The mean values of Alanine amino transferase ALT and Alkaline Phosphatase ALP fed dietary levels of Bioplex zinc were not significantly different among the treatments. Also the values did not follow a particular trend.

Table 5: Serum biochemical parameters of chicks fed dietary levels of Bioplex zinc

	Dietary Zinc Levels (mg/kg)

Parameters	T1 O (Control)	T2 100	T3 200	T4 300	SEM
Total protein (g/dl)	2.30	2.37	2.42	2.48	0.76
Albumin (g/dl)	1.41	1.33	1.52	1.52	0.74
Globulin (g/dl)	0.89	1.04	0.90	0.96	0.10
Albumin/globulin ratio	1.58	1.27	1.69	1.58	0.59
Glucose (mg/dl)	146.15^{a}	143.07^{b}	141.45^{b}	140.20^{b}	1.20
Urea (mg/dl)	6.14	6.09	6.02	6.01	0.09
Creatinine (mg/dl)	1.44	1.40	1.46	1.42	0.62
Cholesterol (mg/dl)	149.23^{a}	136.35^{b}	138.50^{b}	137.58^{b}	0.76
Triglyceride (mg/dl)	110.38^{a}	102.93^{b}	100.45^{b}	101.84^{b}	1.13
AST (i.u/L)	111.89^{a}	106.08^{ab}	105.15^{ab}	100.50^{b}	1.30
ALT (i.u/L)	26.70	25.72	26.73	26.50	0.92
ALP (i.u/L)	24.82	24.88	24.47	25.65	0.14

a,b,c: Means along the same row with different superscripts are significantly different (P<0.05)

ALT: Alanine amino transferase;
AST : Aspartate amino transferase;
ALP; Alkaline phosphatase
*Mitruka and Rawnsley 1981

4.2: STUDY 2: GROWTH, HAEMATOLOGICAL, SERUM PARAMETERS, CARCASS AND RELATIVE ORGAN CHARACTERISTICS OF GROWING COCKERELS FED DIETARY LEVELS OF BIOPLEX ZINC

Table 6: Gross composition (%) of experimental diet fed to growing cockerels.

Feed Ingredients	(%)
Maize	53.59

Palm kernel meal	12.00
Wheat offal	12.00
Groundnuat cake	19.00
Fish meal	1.00
Dicalcium phosphate	1.50
Methionine	0.33
Lysine	0.03
Common Salt	0.30
Vitamin Mineral Premix	0.25
Total	**100.00**
Calculated Nutrients	
Crude protein (%)	18.00
Crude fibre (%)	4.85
Metabolisable energy (kcal/kg)	2737.00
Zinc in the diet (mg/kg)	56.52

Vit+mineral mixture provides per kg of diet: Vitamin A-(50,000,000 i.u) ; Vitamin D_3-(1,600,000i.u) ; Vitamin E(20,000mg) ; Thiamin (2,000 mg) ; Riboflavin (5,000 mg) ; (D-pantothenic acid 10,000 mg) ; Vitamin B6 (3,000 mg) ; Vitamin B_{12} (20 mg) ; Vitamin K (1,000 mg) ; Vitamin C (100,000 mg) ; Nicotinic acid (100,000 mg) ; Folic acid (600mg) ; Biotin (0.5 mg) ; Zinc (40,000 mg) ; Copper (5,000 mg) ; Iodine (200mg) ; Cobalt (250 mg) ; Selenate (125mg) ; Zinc bacitracin (15,000mg) ; Farmers UGf 75,000 mg, Choline chloride (400,000 mg).

Table 7: Proximate composition (g/100g DM) of experimental diets fed to growing cockerels

	Dietary Zinc Levels (mg/kg)				
Nutrients	**T1**	**T2**	**T3**	**T4**	**SEM**
	O (Control)	**100**	**200**	**300**	
Moisture	12.30	12.30	12.50	11.90	0.04

Dry Matter	87.70	87.70	87.50	88.10	0.25
Crude protein	18.34	18.20	18.10	18.42	0.05
Crude Fibre	4.90	5.10	5.27	5.32	0.03
Ether Extract	4.67	5.00	5.10	5.10	0.03
Ash	8.73	8.80	8.80	9.04	0.01
Nitrogen Free Extract	63.36	62.90	62.73	62.12	0.12

4.2.1 Performance characteristics of growing cockerels fed dietary levels of Bioplex zinc

The performance data of the growing cockerels on experimental diets are presented in Table 8. The values of the daily feed intake, daily weight gain, and final body weights though not significantly different (P> 0.05) increased across the treatments as the dietary levels of Bioplex zinc increased with treatment 1 (control) having the least value and treatment 4 having the highest value.

The feed conversion ratio of the birds decreased with increased in the dietary levels of Bioplex zinc. The highest value was obtained for birds fed control treatment (6.64) while the least value was observed for birds on treatment 4 (6.22).

Table 8: Performance characteristics of growing cockerels fed dietary levels of Bioplex zinc

Parameters	Dietary Zinc Levels (mg/kg)				SEM
	T1	T2	T3	T4	
	O (Control)	100	200	300	
Daily feed intake(g/bird)	105.91	106.25	106.40	106.44	0.66
Daily weight gain (g/bird)	15.94	16.08	16.21	17.43	0.18
Final body weight (g/bird)	1496.00	1507.98	1516.98	1541.68	11.31
Feed conversion ratio	6.64	6.61	6.57	6.22	0.05

a,b,c: Means along the same row with different superscripts are significantly different (P<0.05)
SEM: Standard Error of Means

4.2.2 Haematological characteristics of growing cockerels fed dietary levels of Bioplex zinc.

The results of the haematological parameters of the growing cockerels fed dietary levels of Bioplex zinc are presented in Table 9. All the parameters measured namely the packed cell volume, haemoglobin, erythrocytes, MCH and MCHC were not significantly ($P>0.05$) influenced by the dietary levels of Bioplex zinc except the white blood cells and some leukocyte differential counts.

The mean values for the packed cell volume for the experimental growing cockerels were not significantly different as the dietary levels of Bioplex zinc increased. Birds on treatment 4 had the highest value of (27.40%) while the least value was observed for birds on treatment 2 (26.00%). The Haemoglobin and Erythrocytes values though not significantly influenced by the dietary levels of Bioplex zinc followed the same trend as in the packed cell volume. Birds on treatment 4 had the highest values while birds on treatment 2 had the lowest values.

The mean cell volume of the blood of the experimental growing cockerel was significantly influenced by the dietary levels of Bioplex zinc but the numerical differences in the means across the treatments did not follow any particular trend. The mean values for the MCH and MCHC of the experimental growing cockerels were not significantly different for the four treatments. For white blood cell, growing cockerels fed treatments 1 (24.59 $x10^3$/ mm^3), 2 (22.18 $x10^3$/ mm^3 and treatments 3 (20.55$x10^3$/mm^3) were not significantly different while birds on treatment 1 were significantly ($P<0.05$) different from birds on treatment 4 (18.44 $x10^3$/ mm^3) with the least value and was not significantly different to birds on treatments 2 and 3. The mean values obtained for heterophils, and monocytes showed that significant differences existed among the treatments. The mean values of heterophils for growing cockerels fed treatments 1 (28.02%), 3(29.75%) and 4 (28.50%) were not significantly different from one another but birds on treatment 3 were significantly different from birds on treatment 2 (25.25%). As the dietary level of Bioplex zinc increased the lymphocytes, eosinophils and basophils of the experimental growing cockerels were not significantly different.

Table 9: Haematological responses of growing cockerels fed dietary levels of Bioplex zinc

Parameters	Dietary Zinc Levels (mg/kg)				SEM
	T1	T2	T3	T4	
	O (Control)	100	200	300	
Packed cell volume (%)	27.00	26.00	26.80	27.40	0.31
Haemoglobin (g/dl)	9.05	8.33	8.91	9.13	0.12
Erythrocytes (10^6/mm^3)	2.37	2.31	2.70	2.43	0.08
MCV (μ^3)	102.79^b	108.97^a	105.07^{ab}	101.71^b	1.28
MCH (µµg)	35.05	36.63	33.40	36.07	1.36
MCHC (%)	33.30	33.30	33.30	33.30	0.00
White blood cell (10^3/mm^3)	24.59^a	22.18^{ab}	20.55^{ab}	18.44^b	0.69
Heterophils (%)	28.02^{ab}	25.25^b	29.75^a	28.50^{ab}	0.23
Lymphocytes (%)	64.98	69.12	65.07	66.50	1.69
H: L ratio	0.43	0.37	0.46	0.42	0.14
Monocytes (%)	2.60^b	2.60^b	3.00^a	1.20^c	0.36
Eosinophils (%)	4.20	2.63	2.18	3.80	0.43
Basophils (%)	0.20	0.40	0.00	0.00	0.07

a,b,c: Means differently superscripted across the rows are significantly different (P<0.05)
SEM: Standard Error of Means
MCV: Mean Corpuscular Volume
MCH: Mean Corpuscular Haemoglobin
MCHC: Mean Corpuscular Haemoglobin Concentration
* Mitruka and Rawnsley 1981

4.2.3 Serum Biochemical parameters of growing cockerels fed dietary levels of Bioplex zinc.

The serum biochemical parameters of the growing cockerels fed dietary levels of Bioplex zinc is as shown in Table 10. The Total protein, Albumin, Globulin, Albumin/Globulin ratio, Urea, Creatinine, Triglyceride, AST, ALT and ALP of the growing cockerels were not significantly (P> 0.05) influenced as the dietary level of Bioplex zinc increased.

For the total protein values of the experimental growing cockerels, birds on treatments 1(control) (5.44 g/dl), 2 (5.45 g/dl), 3(6.28 g/dl) and 4 (6.32 g/dl) were not significantly (P> 0.05) influenced. The albumin values of the experimental growing cockerels on treatments 1(control) (1.89 g/dl), 2 (1.93 g/dl), 3 (2.34 g/dl) and 4 (2.28 g/dl) were not significantly (P> 0.05) different. The globulin values of the experimental growing cockerels on treatments 1 (control) (3.55 g/dl), 2 (3.53 g/dl), 3 (3.94 g/dl) and 4 (4.04 g/dl) were not significantly (P> 0.05) different. The Albumin/ globulin ratio, urea and creatinine values of the growing cockerels fed treatments 1, 2, 3 and 4 were were not significantly different. The mean values of glucose for the growing cockerels were significantly (P< 0.05) different. Birds on treatments 1 (183.20 mg/dl) and 2 (180.80mg/dl) were not significantly different from each other while birds on treatments 3 (175.33mg/dl) and 4 (174.28mg/dl) were not significantly different but significantly lower (P< 0.05) than birds on treatments 1 and 2. The mean values obtained for cholesterol of experimental growing cockerels were significantly (P< 0.05) different. Birds fed treatments 1 (130.06 mg/dl) and 3 (129.95 mg/dl) were similar and birds on treatments 2 (124.90mg/dl) and 4 (121.73mg/dl) were not significantly different from each other but were significantly (P< 0.05) lower than those birds on treatments 1 and 3. The triglycerides values of the experimental growing cockerels on treatments 1 (control) (107.02 mg/dl), 2 (99.20 mg/dl), 3 (102.54 mg/dl) and 4 (103.98 mg/dl) were not significantly (P> 0.05) different. The AST, ALT and ALP values of the growing cockerels fed treatments 1, 2, 3 and 4 were not significantly different.

Table 10: Serum biochemical parameters of growing cockerels fed dietary levels of Bioplex zinc

Parameters	Dietary Zinc Levels (mg/kg)				SEM
	T1	T2	T3	T4	
	0 (Control)	100	200	300	
Total protein (g/dl)	5.44	5.45	6.28	6.32	0.40
min (g/dl)	1.89	1.93	2.34	2.28	0.16
Globulin (g/dl)	3.55	3.52	3.94	4.04	0.40
Albumin/globulin ratio	0.87	0.99	0.96	0.85	0.20
Glucose (mg/dl)	183.20^{a}	180.80^{ab}	175.33^{b}	174.28^{ab}	1.30
Urea (mg/dl)	3.90	3.50	3.73	3.88	0.30
Creatinine (mg/dl)	0.38	0.39	0.38	0.43	0.01
Cholesterol (mg/dl)	130.06^{a}	124.90^{ab}	129.95^{a}	121.73^{b}	1.09
Triglyceride (mg/dl)	107.02	99.20	102.54	103.98	2.93
AST (i.u/L)	84.24	83.00	81.78	80.86	0.16
ALT (i.u/L)	26.89	27.10	25.72	25.14	0.49
ALP (i.u/L)	24.71	24.84	26.02	26.28	0.39

a,b,c: Means along the same row with different superscripts are significantly different (P<0.05),
SEM: Standard Error of Means
AST : Aspartate amino transferase;
ALT: Alanine amino transferase;
ALP: Alkaline Phosphatase,
* Mitruka and Rawnsley 1981

Table 11: Carcass characteristics of growing cockerels fed dietary levels of Bioplex zinc

Parameters (g)	Dietary Zinc Levels (mg/kg)				SEM
	T1 O (Control)	T2 100	T3 200	T4 300	
Drumstick	223.50^{ab}	212.00^{b}	233.00^{a}	235.20^{a}	1.17
Wings	168.00	160.60	164.40	169.80	1.73
Shank	84.60	80.40	85.40	85.20	1.82
Breast	251.25^{ab}	246.40^{b}	252.00^{ab}	275.25^{a}	2.28
Back	243.25^{b}	260.80^{ab}	265.72^{a}	246.00^{b}	2.66
Head	80.00	77.00	81.40	88.60	2.58
Neck	103.20	92.40	93.00	108.40	3.83
Thigh	216.50^{a}	194.00^{b}	218.60^{a}	221.00^{a}	1.45

a,b,c: Means along the same row with different superscripts are significantly different (P<0.05), SEM: Standard Error of Means

4.2.4 Carcass characteristics of growing cockerels fed dietary levels of Bioplex zinc.

The results of the carcass characteristics of growing cockerels fed dietary levels of Bioplex zinc are shown in Table 11. The mean values for the wings, shank, head and neck of primal cuts of the growing cockerels fed dietary levels of Bioplex zinc were not significantly different among the treatments.

The drumstick values for the experimental growing cockerels were significantly ($P<0.05$) different. Birds on treatments 1 (control) (223.50g), 3 (233.00g) and 4 (235.20g) were not significantly different while birds on treatment 1 was not significantly different from those on treatments 2 (212.00g). The wings values of the experimental growing cockerels on treatments 1 (control) (168.0g), 2 (160.60g), 3 (164.40g) and 4 (169.80g) were not significantly ($P> 0.05$) different. The Shank values of the experimental growing cockerels on treatments 1 (control) (84.60g), 2 (80.40g), 3 (85.40g) and 4 (85.20g) were not significantly ($P> 0.05$) influenced. The mean values for the breast of the growing cockerels fed dietary levels of Bioplex zinc were significantly different among the birds on treatments 1, 2 and 3 were significantly lower ($P< 0.05$) than those birds fed treatment 4 (275.25g). The back values of the growing cockerels fed treatments 1, 2, and 4 were not significantly different from one another. Birds on treatments 1 and 4 were significantly different from birds on treatment 3. The head and neck values of the growing cockerels fed treatments 1, 2, 3 and 4 were not significantly different. The dietary levels of Bioplex zinc had significant effect on the thigh of the experimental growing cockerels. Birds on treatments 1 (216.50g), 3 (218.60g) and 4 (221.00g) were statistically similar and significantly ($P<0.05$) higher than birds fed treatment 2 (194.00g). However the values of the primal cuts did not follow a particular trend across the treatments.

Table 12: Relative weights of some organs of growing cockerels fed dietary levels of Bioplex zinc

	Dietary Zinc Levels (mg/kg)

Parameters	T1 O (Control)	T2 100	T3 200	T4 300	SEM
Live weight (g)	1800.00	1830.00	1890.00	1900.00	0.04
Bled Weight (g)	1690.00	1710.00	1740.00	1830.00	0.02
Plucked weight (g)	1400.00	1420.00	1450.00	1540.00	0.02
Liver (%)	1.82	1.64	1.56	1.60	0.04
Kidney (%)	0.69[a]	0.57[ab]	0.54[b]	0.61[ab]	0.02
Heart (%)	0.53	0.42	0.48	0.50	0.02
Lung (%)	0.58	0.52	0.53	0.51	0.02
Spleen (%)	0.18	0.11	0.11	0.14	0.01
GIT (%)	3.83	3.78	3.44	3.64	0.09
Left testis (%)	0.52	0.45	0.47	0.49	0.02
Right testis (%)	0.52	0.49	0.52	0.49	0.02
Full gizzard (%)	3.46	3.46	3.60	3.19	0.08
Empty gizard (%)	2.35	2.33	2.53	2.30	0.64
Full crop (%)	0.42	0.50	0.38	0.41	0.26
Empty crop (%)	0.25	0.21	0.17	0.21	0.59
Pancreas (%)	0.12	0.12	0.14	0.12	0.005

a,b,c: Means along the same row with different superscripts are significantly different (P<0.05)
SEM: Standard Error of Means;
GIT:Gastro intestinal tract

4.2.5 Relative weights of some organs of growing cockerels fed dietary levels of Bioplex zinc

The relative weights of some internal organs of growing cockerels fed dietary levels of Bioplex zinc are presented in Table 12. The relative mean values for the liver, heart, lungs, spleen, GIT, left and right testes, full and empty gizzard and pancreas of the growing cockerels fed dietary levels of Bioplex zinc were not significantly different among the treatments except the kidney.

The dietary levels of Bioplex zinc significantly ($P<0.05$) influenced the kidney weights among dietary treatments.

4.2.6 Organoleptic characteristics of growing cockerels' meat as influenced by dietary levels of Bioplex zinc

The organoleptic characteristics of growing cockerels' meat as influenced by dietary levels of Bioplex zinc are presented in Table 13. The mean values for aroma, colour, flavor and tenderness of the growing cockerels fed dietary levels of Bioplex zinc were not significantly different. The aroma mean values of the experimental growing cockerels on treatments 1 (control) (33.67%), 2 (34.33%), 3 (38.67%) and 4 (31.00%) were not significantly ($P> 0.05$) different. The colour mean values of the experimental growing cockerels on treatments 1 (control) (50.33%), 2 (56.33%), 3 (52.67%) and 4 (58.33%) were not significantly ($P> 0.05$) influenced. The flavour mean values of the experimental growing cockerels on treatments 1 (control) (41.33%), 2 (38.67%), 3 (42.33%) and 4 (42.00%) were not significantly ($P> 0.05$) different. The dietary levels of Bioplex zinc significantly ($P<0.05$) influenced the juiciness among the treatments. Birds on treatments 1(41.67%) and 2 (45.33%) were not significantly different while birds on treatments 2, 3 (52.33%) and 4 (50.67%) were not significantly different and higher ($P<0.05$) to birds on treatment 1. The tenderness values of the growing cockerels fed treatments 1, 2, 3 and 4 were not significantly different. The taste panel rating showed that acceptability was birds on treatment 3, and lowest in birds on treatment 1. Although there were no significant differences in the overall acceptability amongst the dietary treatments.

Table 13: Organoleptic Characteristics of Growing Cockerels meat as influenced by dietary levels of Bioplex Zinc

Parameters (%)	Dietary Zinc Levels (mg/kg)				SEM
	T1	T2	T3	T4	
	0 (Control)	100	200	300	
Aroma	33.67	34.33	38.67	31.00	2.07
Colour	50.33	56.33	52.67	58.33	2.02
Flavour	41.33	38.67	42.33	42.00	2.94
Juiciness	41.67^{b}	45.33^{ab}	52.33^{a}	50.67^{a}	1.52
Tenderness	57.00	56.00	61.33	57.33	1.91
Oveerall Acceptability	50.67	52.67	55.33	54.00	1.20

a,b,c: Means along the same row with different superscripts are significantly different (P<0.05)
SEM: Standard Error of Means

4.3. STUDY 3: PERFORMANCE, NUTRIENT DIGESTIBILITY, ZINC RETENTION AND HISTOPATHOLOGICAL EXAMINATION OF COCKS FED DIETARY LEVELS OF BIOPLEX ZINC

4.3.1 Proximate Composition

The proximate composition of the experimental diets fed to cocks is presented in Table 7.

The proximate composition (g/100gDm) of faecal sample of cocks fed dietary levels of Bioplex zinc is shown in Table 14 ; The crude protein ranged between 21.84% (treatment 1) and 22.87% (treatment 4), the crude fibre from 16.84% (treatment 3) to 18.78% (treatment 1). The ether extract ranged from 10.00% (treatment 4) to 11.50% (treatment 1). The Ash values increased from 13.06% (treatment 1) to 14.18% (treatment 4). The Nitrogen free extract values increased from 34.83% (treatment 1) to 35.98% (treatment 4)

Table 14: Proximate composition (g/100g DM) of faecal of cocks fed dietary levels of Bioplex zinc

Nutrients	Dietary Zinc Levels (mg/kg)				SEM
	T1 O (Control)	T2 100	T3 200	T4 300	
Moisture	12.15	11.40	11.52	10.64	0.03
Dry Matter	87.85	88.60	88.48	89.36	0.35
Crude protein	21.84	22.58	22.62	22.87	0.04
Crude Fibre	18.78	18.42	16.84	16.97	0.05
Ether Extract	11.50	10.50	10.50	10.00	0.02
Ash	13.06	13.54	14.09	14.18	0.02
Nitrogen Free Extract	34.83	34.96	35.95	35.98	0.03

Table 15: Performance Characteristics of Cocks fed dietary levels of Bioplex zinc

Parameters	Dietary Treatments (mg/kg)				SEM
	T1	T2	T3	T4	
	0 (Control)	100	200	300	
Daily feed intake(g/bird)	158.17^{c}	161.62^{c}	165.12^{b}	`174.50^{a}	0.49
Daily weight gain (g/bird)	15.89^{c}	16.54^{b}	16.98^{b}	18.11^{a}	0.20
Final body weight (g/bird)	2603.60^{b}	2645.60^{ab}	2699.00^{a}	2772.40^{a}	9.58
Feed conversion ratio (FCR)	9.95	9.77	9.70	9.64	0.12

a,b,c: Means differently superscripted across the rows are significantly different (P<0.05)

SEM: Standard Error of Means

4.3.3 Haematological responses of Cocks fed dietary levels of Bioplex zinc

The results of the haematological parameters of cocks are presented in Table 16.

The mean corpuscular volume MCV, mean corpuscular haemoglobin MCH, white blood cells and heterophils of the blood were signfiicnatly influenced as the dietary levels of Bioplex zinc increased.

The mean cell volume and the mean corpuscular haemoglobin of the blood of the experimental cocks were significantly altered by the dietary levels of Bioplex zinc but the differences in the mean across the treatments did not follow any particular trend. The white blood cells values for the experimental cocks fed treatments 2 (14.68 x 10^3/mm^3), 3 (14.30 x 10^3/mm^3) and 4 (14.12 x 10^3/mm^3) were not significantly different and were significantly lower ($P<0.05$) than those birds fed treatment 1 (control) (16.88 x 10^3/mm^3). The mean values for the heterophils components of the leukocytes of the birds fed across the treatments were statistically similar but significantly different from birds on treatment 3. The values did not follow a particular trend. However, the mean values for the packed cell volume, haemoglobin, erythrocytes, MCHC, lymphocytes, monocytes, eosinophils, and basophils of the experimental cocks fed dietary levels of Bioplex zinc were not significantly different across the four treatments. This suggests that the blood of the birds had an appreciable oxygen carrying capacity.

4.3.4 Serum Biochemical Parameters of Cocks fed dietary levels of Bioplex zinc

The result of the serum biochemical parameters of cocks fed dietary levels of Bioplex zinc are shown in Table 17. The total protein albumin, globulin, albumin/globulin ratio, urea, creatinine AST, ALT and ALP of the experimental cocks were not significantly ($P>0.05$) influenced as the dietary levels of Bioplex zinc increased.

The glucose, cholesterol and triglyceride values in the serum of birds fed dietary levels of Bioplex zinc were significantly influenced with birds on treatment 1 (control) having the highest values in the three parameters. However differences in the means across the treatments did not follow any particular trends.

Table 16: Haematological responses of cocks fed dietary levels of Bioplex zinc

	Dietary Zinc Levels (mg/kg)				
Parameters	**T1**	**T2**	**T3**	**T4**	**SEM**

	0 (Control)	100	200	300	
Packed cell volume (%)	32.20	35.40	32.40	34.50	0.83
Haemoglobin (g/dl)	11.73	12.47	11.98	11.93	0.27
Erythrocytes (10^6/mm^3)	2.77	2.67	2.80	2.93	0.13
MCV (μ^3)	129.36^b	149.78^a	142.8^b	131.77^c	1.25
MCH (µµg)	43.12^b	48.41^a	43.43^b	42.46^b	0.76
MCHC (%)	33.30	33.30	33.30	33.30	0.00
White blood cell (10^6/mm^3)	16.88^a	14.68^b	14.30^b	14.12^b	0.85
Heterophils (%)	25.75^b	24.60^{ab}	23.50^b	27.60^a	0.49
Lymphocytes (%)	65.80	68.20	69.20	66.58	1.41
H: L ratio	0.39	0.36	0.34	0.41	0.25
Monocytes (%)	3.40	3.60	2.40	3.40	0.31
Eosinophils (%)	4.45	2.40	2.24	2.22	0.46
Basophils (%)	0.60	0.00	0.00	0.40	0.13

a,b,c: Means along the same row with different superscripts are significantly different ($P<0.05$)
SEM: Standard Error of Means
MCV: Mean Corpuscular Volume
MCH: Mean Corpuscular Haemoglobin

Table 17: Serum biochemical parameters of cocks fed dietary levels of Bioplex zinc

	Dietary Zinc Levels (mg/kg)				
	T1	T2	T3	T4	SEM
Parameters	**O (Control)**	**100**	**200**	**300**	

Total protein (g/dl)	7.17	8.03	8.23	8.52	0.25
Albumin (g/dl)	5.50	4.51	5.89	5.52	0.14
Globulin (g/dl)	1.67	3.52	2.34	3.00	0.18
Albumin/globulin ratio	3.80	2.01	2.66	2.01	0.18
Glucose (mg/dl)	225.50[a]	218.30[b]	222.38[b]	219.37[b]	1.93
Urea (mg/dl)	1.83	1.64	1.67	1.58	0.08
Creatinine (mg/dl)	0.67	0.64	0.63	0.58	0.03
Cholesterol (mg/dl)	125.20[a]	120.92[ab]	109.38[b]	119.42[ab]	3.73
Triglyceride (mg/dl)	110.22[a]	101.70[ab]	97.24[b]	102.46[ab]	1.07
AST (i.u/L)	99.48	93.32	93.93	96.08	0.27
ALT (i.u/L)	27.84	26.09	25.84	27.24	0.72
ALP (i.u/L)	39.60	41.07	40.85	42.76	0.51

a,b,c: Means along the same row with different superscripts are significantly different (P<0.05)
SEM: Standard Error of Means
AST : Aspartate amino transferase;
ALT: Alanine amino transferase;
ALP; Alkaline Phosphatase

* Mitruka and Rawnsley 1981

4.3.5 Histopathology of selected organs in cocks

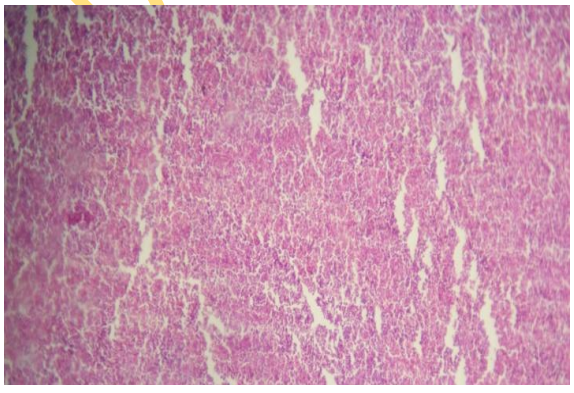
The results of the histopathology report of selected organs of cocks fed dietary levels of Bioplex zinc is as shown in Table 18. No visible lesions were observed in the spleen, liver and testes of the experimental cocks across the treatments.

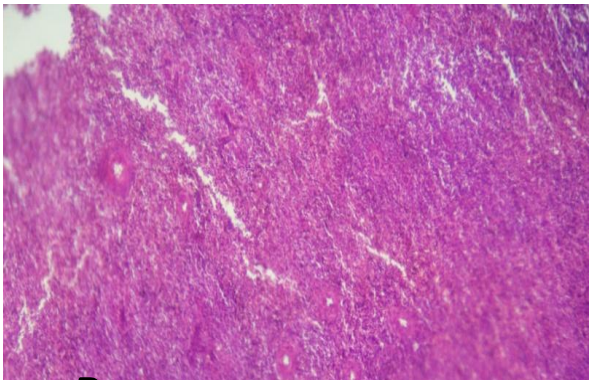
Renal congestions were observed on the kidney of 10% of the cocks sacrified in treatment 4 while the birds on treatments 1, 2 and 3 had no congestions in their kidney. No cellular infiltration was observed in the testes of cocks across the treatments.

Table 18: Histopathological reports of selected organs of cocks fed dietary levels of Bioplex zinc

	Dietary Zinc Levels (mg/kg)			
	T1	**T2**	**T3**	**T4**
Organs	**O (Control)**	**100**	**200**	**300**

Spleen	No visible lesions	No visible lesions	No visible lesions	No visible lesions
Liver	No visible lesions	No visible lesions	No visible lesions	No visible lesions
Kidney	No visible lesions	No visible lesions	No visible lesions	Renal congestions
Testes	No visible lesions	No visible lesions	No visible lesions	No visible lesions



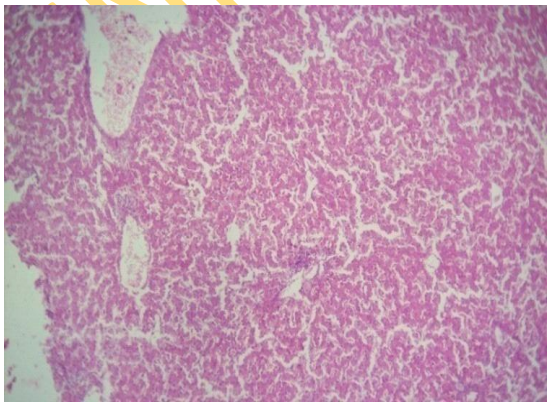


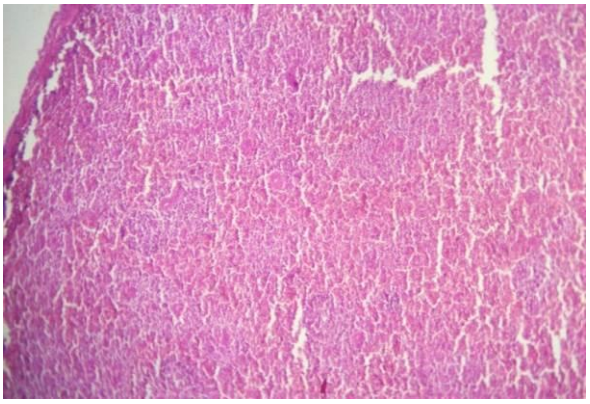
A Control 0 mg/kg (T1) **B 100 mg/kg (T2)**

C 200 mg/kg (T3) **D 300 mg/kg (T4)**

Figure 2: Micrographs of the spleen of cocks fed dietary levels of Bioplex zinc

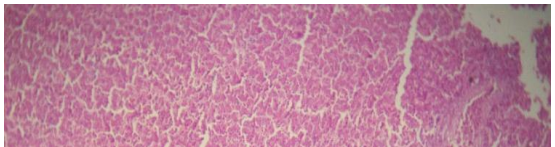
A – D: No Visible lessions seen (Magnification x 400)

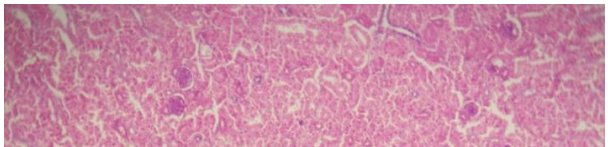




A Control 0 mg/kg (T1)

B 100 mg/kg (T2)



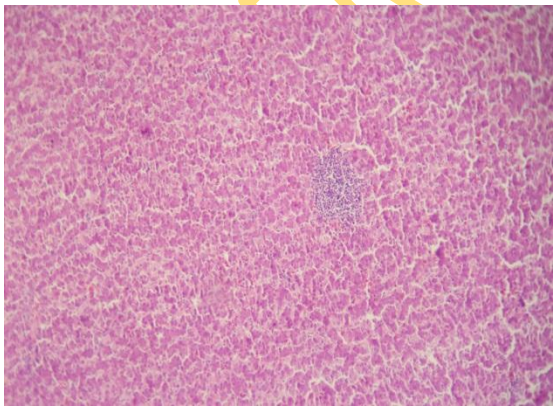


C **200 mg/kg (T3)**

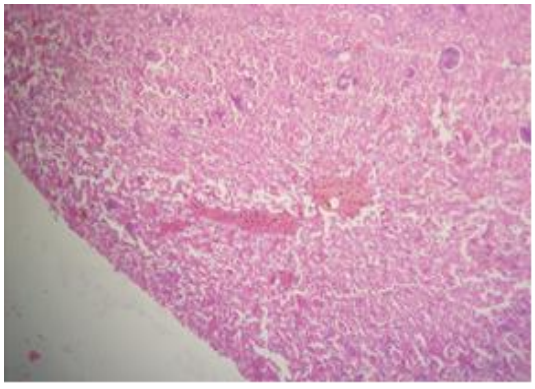
D **300 mg/kg (T4)**

Figure 3: Micrographs of the livers of cocks fed dietary levels of Bioplex zinc

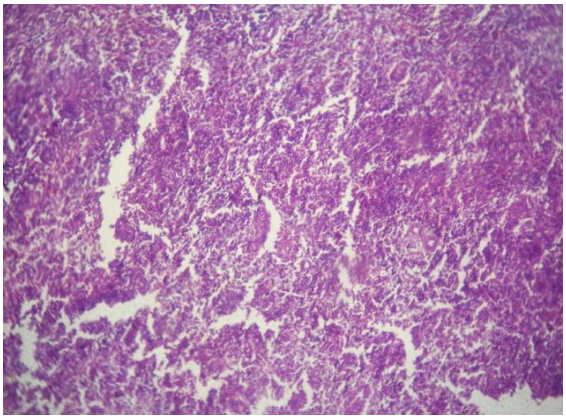
A –D: No visible lesions seen (Mag x 400)



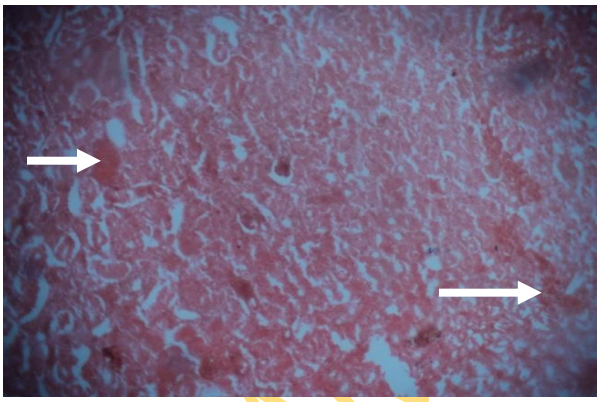
A **Control 0 mg/kg (T1)**



B **100 mg/kg (T2)**



C **200 mg/kg (T3)**

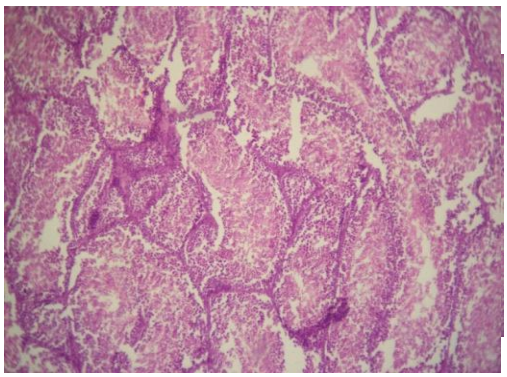


D **300 mg/kg (T4)**

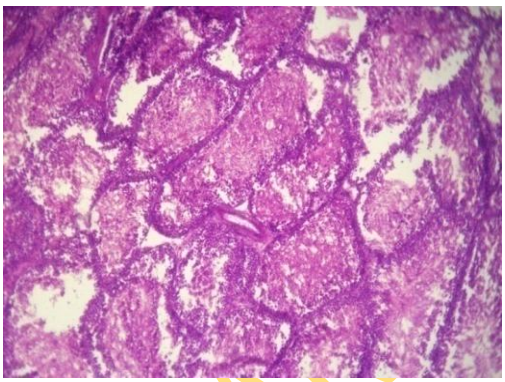
Figure 4: Micrographs of Kidneys of cocks fed dietary levels of Bioplex zinc

A-C: No visible lesions seen

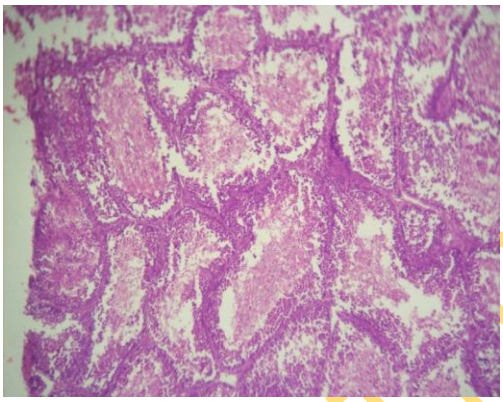
D: Renal congestions (Mag x400)



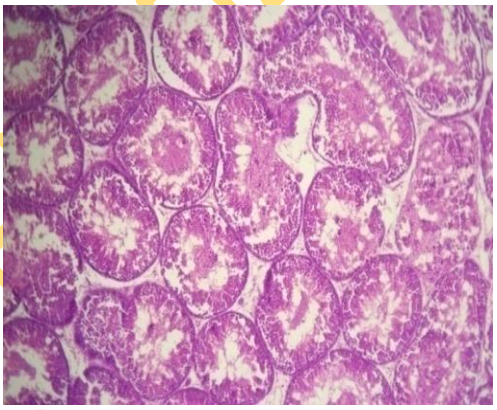
A **Control 0 mg/kg (T1)**



B **100 mg/kg (T2)**



C **200 mg/kg (T3)**



D **300 mg/kg (T4)**

Figure 5: Micrographs of the testes of cocks fed dietary levels of Bioplex zinc
A – D: No visible lessions seen (Magnification x 400

4.3.6 Apparent nutrient digestibility

The apparent nutrient digestibility values of the experimental diets are shown in Table 21. The mean dry matter digestibility value for cocks fed dietary levels of Bioplex zinc was not significantly influenced (P>0.05). Birds on treatment 1 (control) (72.77%) had the highest value while birds on treatment 3 (69.14%) had the lowest value. The mean digestibility values for crude protein increased significantly (P<0.05) with increase in the dietary levels of Bioplex zinc. Birds on treatments 2 (65.23%) and 3 (65.55%) were not significantly different while birds on treatment 1 (control) (61.10%) was significantly different from birds on treatment 4 (67.95%). For crude fibre, birds on treatment 3 (53.16%) had the highest digestibility value while birds on treatment 1 (control) (50.79%) had the lowest value, for ether extract digestibility, the mean values increased as the dietary levels of Bioplex zinc increased. The digestibility of ash significantly increased with increase in the dietary levels of Bioplex zinc. Birds on treatments 1 (52.25%), 2 (53.00%) and 3 (55.04%) were not significantly different but significantly lower (P<0.05) than birds on treatment 4 (60.30%). Nitrogen free extract digestibilities though not significantly different decreased in values and did not follow a particular trend with respect to the dietary levels of Bioplex zinc

4.3.7 Zinc utilization by cocks fed dietary levels of Bioplex zinc

The utilization of zinc by cocks fed dietary levels of Bioplex zinc is as shown in Table 22. The daily feed intake, zinc intake, zinc losses, zinc retained and zinc retention (% of intake) were all significantly (P<0.05) influenced with increase in the dietary levels of Bioplex zinc.

The mean values of the zinc intake of the experimental cocks were significantly higher as the dietary levels of Bioplex zinc increased. Birds on treatment 4 (51.14 mg/kg) had the highest mean value with birds on treatment 1 (8.94 mg/kg) having the lowest: the zinc retained mean values for the experimental cocks followed the same trend with those of the zinc intakes. The zinc losses values were significantly influenced as the dietary

levels of Bioplex zinc increased. Birds on treatment 1 (5.82 mg/kg) had the lowest values and treatment 4 (15.91 mg/kg) with the highest values. The zinc retention (% of intake) mean values were significantly influened by the dietary levels of Bioplex zinc and increased across the treatments. Birds on treatment 1 (34.96 mg/kg) had the lowest values and treatment 4 (69.09 mg/kg) with the highest values. .

Table 19: Apparent nutrient digestibility (%) of cocks fed dietary levels of Bioplex zinc

Nutrients (%)	Dietary Zinc Levels (mg/kg)				SEM
	T1 O (Control)	T2 100	T3 200	T4 300	
Dry Matter	70.05	70.79	69.14	72.89	1.52
Crude protein	61.10^{b}	65.23^{ab}	65.55^{ab}	67.95^{a}	0.78
Crude Fibre	50.79	51.10	53.16	52.70	0.05
Ether Extract	36.36	37.12	37.99	39.48	1.01
Ash	52.25^{b}	53.00^{b}	55.04^{ab}	60.30^{a}	1.03
Nitrogen Free Extract	84.76	82.95	82.63	84.86	0.57

a,b,c: Means differently superscripted across the rows are significantly different ($P<0.05$)
SEM: Standard Error of Means

Table 20: Zinc utilisation by cocks fed dietary levels of Bioplex zinc

	Dietary Zinc Levels (mg/kg)				
	T1	**T2**	**T3**	**T4**	**SEM**
Parameters					
(md/g)	**0 (Control)**	**100**	**200**	**300**	
Daily feed intake	158.17^{c}	161.62^{b}	165.12^{b}	174.50^{a}	0.49
Daily zinc intake	8.94^{d}	16.06^{c}	33.22^{b}	51.14^{a}	1.34
Daily zinc losses	5.82^{c}	9.00^{b}	15.86^{a}	15.91^{a}	0.92
Zinc retained	3.12^{d}	7.06^{c}	17.36^{b}	35.23^{a}	1.76
Zinc retention (%)	34.96^{d}	43.94^{c}	52.27^{b}	69.09^{a}	1.09

a,b,c: Means along the same row with different superscripts are significantly different (P<0.05)
SEM: Standard Error of Means

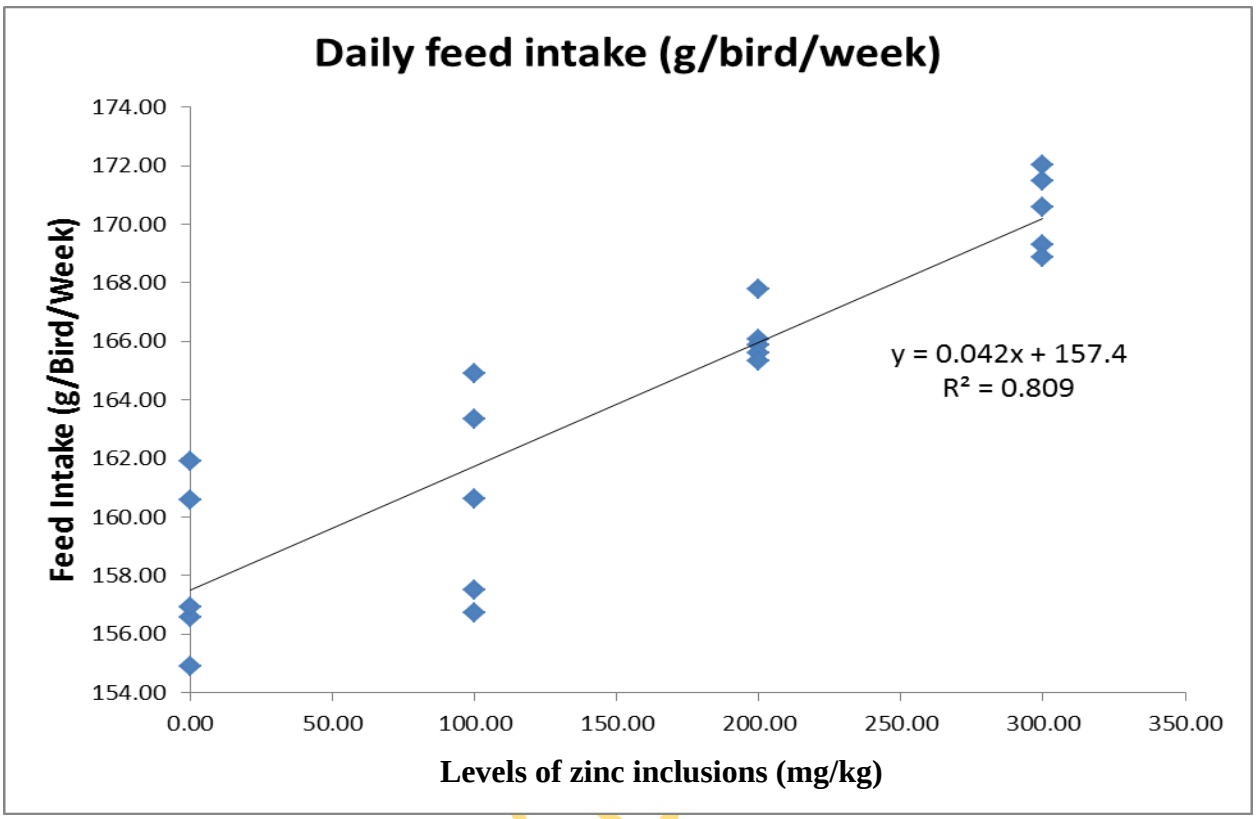


Figure 6: Linear regression coefficient of daily feed intake and dietary levels of Bioplex zinc

The daily feed intake was plotted against levels of zinc inclusions using linear regression technique.

The slope of the graph represented the daily feed intake of Bioplex zinc.

Y= c + mX; where Y= Daily feed intake of Bioplex zinc; c= intercept; m= regression slope (% daily feed intake); X= levels of zinc inclusions (mg/kg).

DFI Y= 0.042X +157.4 R^2 =0.0809

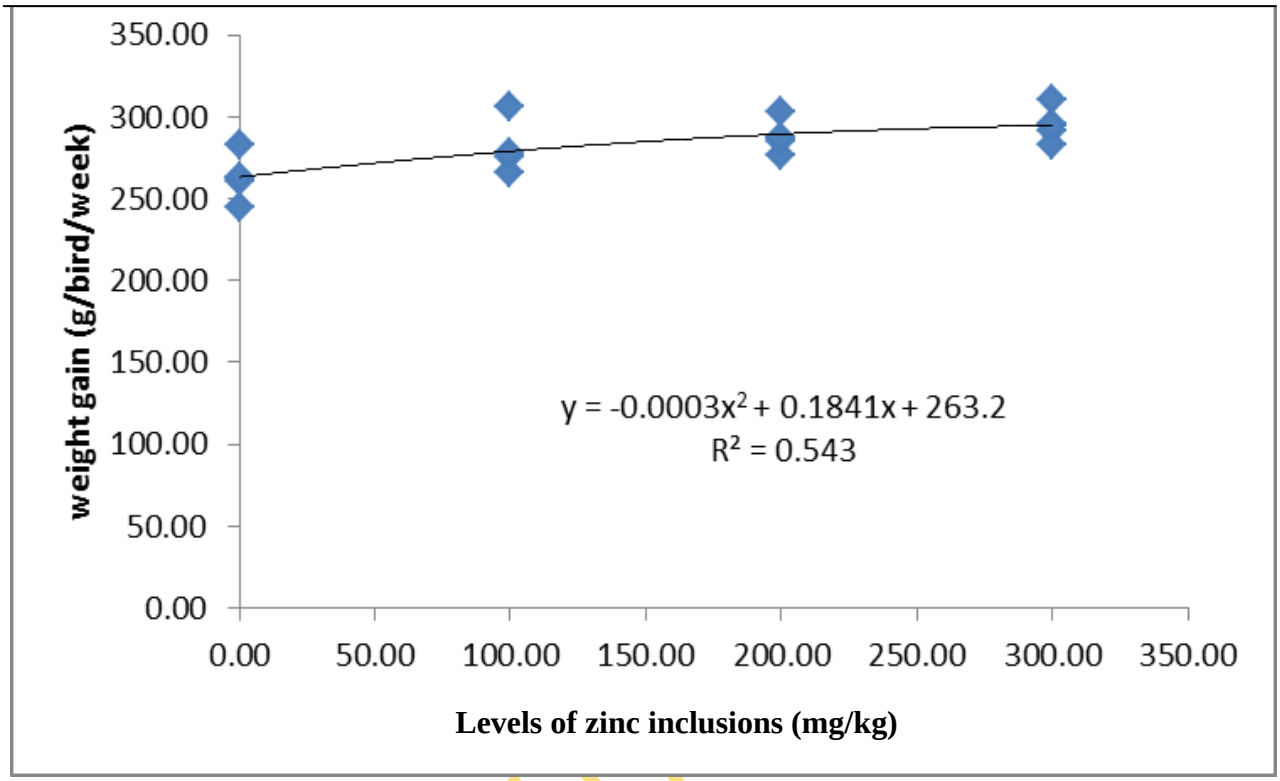


Figure 7: Polynomial regression coefficient of weight gain and dietary levels of Bioplex zinc

The weight gain was plotted against levels of zinc inclusions using polynomial regression technique.

The slope of the graph represented the Bioplex zinc.

Y= c + mX; where Y= weight gain of cocks fed Bioplex zinc; c= intercept; m= regression slope (% weight gain); X= levels of zinc inclusions (mg/kg).

WG $Y= -0.0003X^2 + 0.118444X + 263.3$ $R^2 =0.543$

4.4. STUDY 4: SEMEN CHARACTERISTICS OF COCKS FED DIETARY LEVELS OF BIOPLEX ZINC

4.4.1 Age at puberty

The result showed that dietary Bioplex zinc significantly influenced the age at attaintment of puberty of the cocks. The ages at puberty for the cocks in the control diet, Treatments 1, 2, 3 and 4 were 136±0.70, 131±0.64, 129±0.71 and 126± 0.72 days respectively.

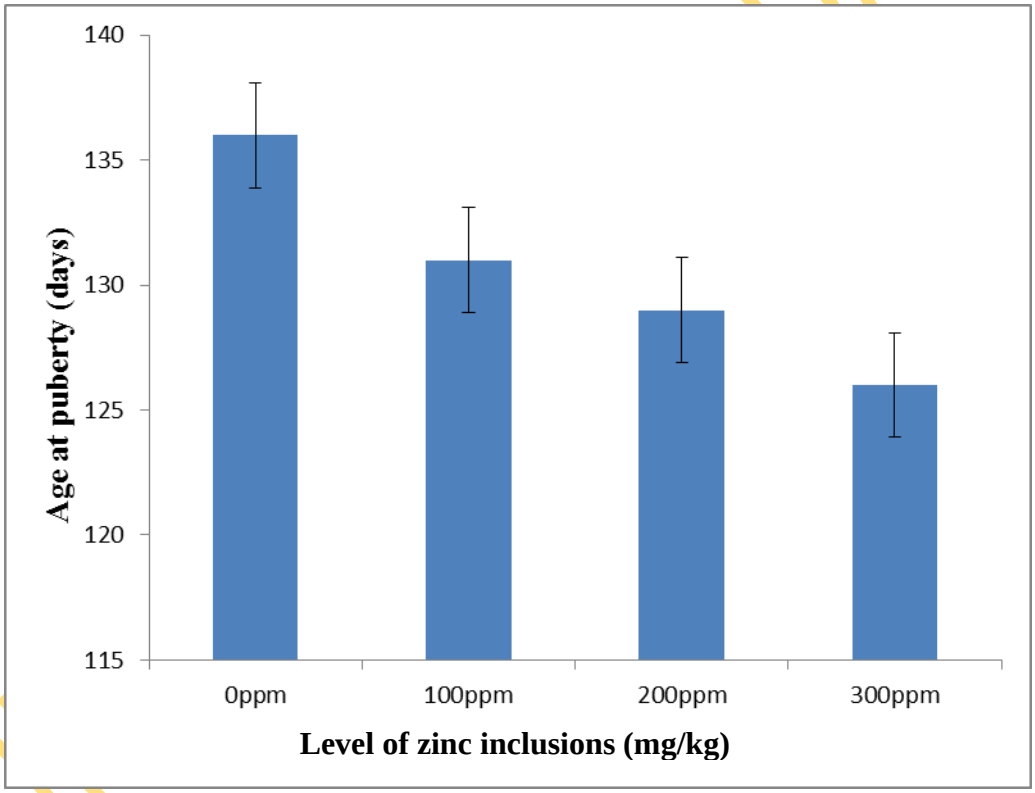


Figure 8: Age at puberty (days) of cocks fed dietary levels of Bioplex zinc

4.4.2 Weight at puberty and semen Characteristics

The results of the effects of dietary levels of Bioplex zinc on the body weights and semen characteristics of cocks are shown in Table 21.

The sperm motility, live to death ratio of the spermatozoa and the sperm concentration were significantly influenced by the dietary levels of Bioplex zinc. Cocks on treatment 4 had the highest body weight (2.56 kg) at puberty while the least weight was observed for cocks on treatment 1 (2.40 kg). The variations in the body weights mean values across the treatments were consistent. The percentage progressive motility of spermatozoa significantly increased with increase in the dietary levels of Bioplex zinc. Cocks on treatment 4 had the highest sperm motility (86.05 %) while the lowest was recorded for cocks on treatment 1 (80.07 %). The pattern of increase in live to dead ratio of the spermatozoa followed the same trend with the sperm motility. Percentage incidence of dead spermatozoa was highest in the cocks on treatment 1 (9.33%) compared to treatment 4 (2.67 %) which had the least. The semen concentration values were significantly ($P<0.05$) influenced, cocks on treatments 2 (0.32×10^9), 3 (0.33×10^9) and 4 (0.37×10^9) were not significantly different while cocks on treatment 4 were significantly different from cocks on treatment 1 (0.29×10^9).The semen volume values (0.03ml) were the same across the treatments. The pH value ranged between 6.52 (treatment 1) and 6.67 (treatment 2). The colour, consistency and mass activity were the same across the treatments. Semen from cocks across the treatments demonstrated turbulent swirling in their mass activity.

Table 21: Weight at puberty and semen characteristics of cocks fed dietary level of Bioplex zinc

Parameter	Dietary Zinc levels (mg/kg)				SEM
	T1	T2	T3	T4	
	0 (Control)	100	200	300	
Body Weight (Kg)	2.40	2.44	2.47	2.56	0.08
Sperm Mortality (%)	80.07b	85.00a	85.20a	86.05a	0.20
Sperm Live / Dead ratio	90.67^{b}	96.73^{a}	96.90^{a}	97.33^{a}	0.38
Semen Colour	Creamy White	Creamy White	Creamy White	Creamy White	-
Semen Volume (mL)	0.30	0.30	0.30	0.30	0.00
Sperm Concentration ($X10^{9}$ mL)	0.29^{b}	0.32^{ab}	0.33^{ab}	0.37^{a}	0.06
Semen pH	6.52	6.67	6.61	6.57	0.02
Semen Consistency	Thin	Thin	Thin	Thin	-
Mass Activity	+++	+++	+++	+++	-

a,b,c: Means along the same row with different superscripts are significantly different (P<0.05)
SEM: Standard Error of Means

4.4.3. Assessment of sperm abnormalities of cocks fed dietary levels of Bioplex zinc

Assessment of sperm abnormalities of the experimental cocks fed dietary levels of Bioplex zinc are presented in Table 22. Sperm abnormalities such as headless tails, tailless heads, looped tails, coiled tails, bent tails, and bent midpiece were not significantly influenced by the Bioplex Zinc among the treatments except the the rudimentary tail (RT) and curved mid-piece (CMP). The mean values of rudimentary tail of cocks on treatments 1 (0.46 %) 2 (0.51 %) and 4 (0.45 %) were not significantly different but values of cocks on treatment 2 were significantly different from cocks on treatment 3 (0.39 %). The mean values for cocks with curved mid-piece were significantly influenced by the dietary levels of Bioplex zinc however there was no particular trend across the treatment. The total abnormal sperm cells decreased as the dietary level of Bioplex zinc increased across the treatments.

4.4.4 Testicular Morphometry

The testicular and epididymal characteristics of cocks fed dietary levels of Bioplex zinc is as shown in Table 23. There were no significant differences in the testes weight, testes volume, testes density, epididymal weight and tunica albugineal weight of the cocks across the treatments. However, the weights of the left testis, left epididymus and left tunica albuginea appeared to be superior to the weights of those on the right.

Table 22: Assessment of sperm abnormalities of cocks fed dietary levels of Bioplex zinc

	Dietary Zinc levels (mg/kg)				
Parameters (%)	**T1**	**T2**	**T3**	**T4**	**SEM**
	0 (Control)	**100**	**200**	**300**	
Tailless head	1.01	0.84	0.90	0.70	0.02
Headless tail	1.14	1.18	1.12	1.04	0.02
Rudimentary tail	2.27^{ab}	2.44^{a}	2.20^{ab}	1.92^{b}	0.01
Bent tail	1.24	1.28	1.34	1.29	0.02
Curved tail	1.51	1.30	1.29	1.07	0.02
Curved mid-piece	2.26^{a}	2.04^{ab}	1.96^{ab}	1.86^{b}	0.02
Bent mid-piece	1.58	1.54	1.49	1.54	0.03
Looped tail	0.34	0.34	0.36	0.35	0.01
Total Abnormal cells	11.35	10.96	10.66	9.77	0.01

a,b,c: Means differently superscripted across the rows are significantly different (P<0.05)
SEM: Standard Error of Means

Table 23: Testicular and Epididymal characteristics of cocks fed dietary levels of Bioplex zinc

Traits		Dietary Zinc levels (mg/kg)				SEM
		T1	T2	T3	T4	
		0 (control)	100	200	300	
Body weight (kg)	2.40	2.44	2.47	2.57	0.08	
Testis weight (g)						
Right (R)	12.60	14.40	12.40	13.60	1.72	
Left (L)	15.20	15.00	12.80	14.50	1.79	
Paired (R +L)	27.80	29.40	25.20	28.10	3.79	
Testis volume (cm^3)						
Right (R)	11.60	13.80	10.60	15.40	2.53	
Left (L)	14.80	12.00	13.60	14.40	3.14	
Paired (R+L)	26.40	25.80	24.20	29.80	5.02	
Testis Density (g/cm^3)						
Right (R)	1.09	1.04	1.16	0.88	0.03	
Left (L)	1.02	1.25	0.94	1.01	0.02	
Paired (R+L)	2.11	2.27	2.10	1.89	0.17	
Epididymal weight (g)						
Right (R)	1.94	1.78	1.98	2.03	0.35	
Left (L)	2.05	2.10	2.17	2.23	0.39	
Paired (R+L)	3.99	3.88	4.15	4.26	0.78	
Tunica albuginea weight (g)						
Right (R)	0.74	0.68	0.70	0.74	0.15	
Left (L)	0.79	0.72	0.81	0.81	0.37	
Paired (R+L)	1.50	1.40	1.51	1.58	0.09	

SEM- Standard Error of Means

CHAPTER 5

DISCUSSION

5.1 STUDY I: PERFORMANCE OF CHICKS FED DIETARY LEVELS OF BIOPLEX ZINC

5.1.1 Performance characteristics of cockerels

The daily feed intake (DFI) of the experimental chicks increased as the Bioplex zinc levels increased in the diets. This increase in daily feed intake of the chicks may probably be due to the increase in feed consumption with enhanced zinc utilization occasioned by the high-bioavailability of dietary Bioplex zinc as reported by Ao *et al*. (2006) that zinc added to diets/rations at substantially higher levels than the animal requirements have shown growth promoting effect in birds. The result from the study also agreed with the reports of Huang *et al*. (2007) who observed significant difference in daily feed intake of broiler chicks fed dietary levels of zinc for 21 days. This result however differs from the work of Tactacan (2001) who reported no significant difference in the feed consumption of broilers fed proteinated supplements. The daily weight gain of the experimental chicks fed dietary levels of Bioplex zinc significantly increased with increase in the levels of Bioplex zinc. The daily weight gain of the birds in the study is directly related to the daily feed intake. This result is similar to that reported by Mohanna and Nys (1999), who found that chicks body weight gain and food intake increased with the dietary Zn content. Similar results were obtained by Huang *et al*. (2007). On the other hand, the result is contrary to the findings of Leeson (2003) and Rossi *et al.* (2007) who reported that using trace minerals with greater bioavailability (Bioplex ™ trace minerals) did not influence body weight gain and had little effect on feed efficiency of broilers. The final body weight was significantly different and

increased as the dietary Bioplex zinc increased among the diets. This result is in support of Leeson (2003) and Ao *et al*. (2006) who reported the growth enhancing capability of Bioplex zinc in chicks. FCR indicates the quantity of feed consumed to gain a unit weight of flesh which is an indication of better utilization of the nutrients in the diets due to the high biological values of Bioplex zinc.

It has been reported that growth retardation is universally observed in Zn deficiency, perhaps because of impairment of nucleic acid biosynthesis and zinc deficiency also results in impaired amino acid utilization or protein synthesis (O'Dell, 1981). Loss of appetite is one of the first signs of deficiency; with poor growth, it may be the only overt sign of a mild deficiency. Severely Zn-restricted rats eat as little as one third that of ad libitum-fed controls, with significantly less fluid. Reduced feed intake may relate to the role of Zn in taste (Hambidge *et al.*, 1986). Subsequent to the onset of deficiency and anorexia, there is loss of taste acuity (Miller *et al.*, 1979). This result is in agreement with those of Tactacan (2001) and Nollet *et al*. (2007) who reported that in the starter period feed conversion rate tended to improve in broilers fed organically complexed mineral diet.

5.1.2 Haematological Parameters

Church *et al.* (1984) reported that dietary components have measurable effects on blood constituents which provide a valuable medium for clinical investigation and assessment of nutritional status of human beings and animals. In this study the mean values obtained for the White blood cells, Heterophils, Lymphocytes, Monocytes and Eosinopils were significantly influenced by the dietary levels of Bioplex zinc while the values obtained for Packed cell volume (PCV), Haemoglobin, Erythrocytes, Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were all statistically similar among the treatments. The results of the packed cell volume (PCV) of the experimental chicks were within the normal physiological range (26-45.2%) reported by Mitruka and Rawnsley (1981) for normal chickens. A PCV of less than 26.0% indicates aneamia while one greater than 45.2% suggests dehydration. From this study, birds fed dietary levels of Bioplex zinc had

normal concentration of the red blood cells. The result is at variance with the findings of Levengood *et al.* (2000) who recorded a low PCV of 22% for ducks fed Bioplex zinc.

The haemoglobin concentration of the chicks were not significantly altered by the dietary levels of Bioplex zinc and the mean values within the physiological range of 7.5-13.1 g/dl reported by Mitruka and Rawnsley (1981) for normal chickens. The results showed that the chicks were not anemic. The erythrocytes components of the blood of the experimental chicks were not significantly influenced with increase in the levels of Bioplex zinc. Although the erythrocyte values for all the treatments were within the physiological range of 2.90-4.10x10^6/mm^3 for normal male chickens reported by Mitruka and Rawnsley (1981). It can be concluded that the dietary treatments did not adversely influenced erythrogenesis (synthesis of RBC) and the experimental chicks may not have suffered from temporary or progressive polycythemis (increase in the number of RBC) caused by the dietary levels of Bioplex zinc. These results are consistent with the findings of Akbari *et al.* (2008) and Donmez *et al*. (2002) who reported no significant differences in the RBC and Hb in broiler chickens. On the other hand, the results constrasted with Levengood *et al*. (2000) who reported a higher value of RBC (15.8 x10^6/µL) in mallards fed zinc.

The MCV of the blood of all the experimental chicks among the treatments were statistically similar and were within the physiological range 100.0-128.0$µ^3$ reported by Mitruka and Rawnsley (1981) for normal male chickens. An MCV below 100.0$µ^3$ indicates microcytosis where a value above 128.0$µ^3$ indicates macrocytosis. With respect to the results, MCV values obtained in this study showed that the red blood cells of the experimental chicks can be classified as normal. The mean corpuscular haemoglobin (MCH) values were within the range of normal physiological values (25.4-33.4 µµg) reported for male chickens (Mitruka and Rawnsley, 1981). This may probably suggest that all blood samples from the chicks across the treatments had similar red blood cell sizes. The mean corpuscular haemoglobin concentration (MCHC) values were not significantly different across the treatments. The values were within the normal physiological range (25.3-32.5) for male chickens reported by Mitruka and Rawnsley

(1981). The results were consistent with those of Aksu *et al.* (2010). It can be inferred that the birds had normal chromastosis.

The white blood cells (Leukocytes) of the experimental chicks was significantly lowered by the dietary levels of Bioplex zinc across the treatments and within the range of normal male chickens reported by Mitruka and Rawnsley (1981). Since high level of white blood cells in the blood is an indication of pathogenic infection or presence of toxic substances, therefore the result was an indication of the positive immune response activities on the experimental birds. Moreever, zinc is a co-factor in the production of lymphocytes which are precursors for thymocyte cells (Tcells) essential in immunological processes. The lymphocyte components of the white blood cells were significantly increased as the dietary levels of Bioplex zinc increased and the values were within the range of normal chickens reported by Mitruka and Rawnsley (1981). Since the principal function of lymphocytes is in relationship to its immunological activities, the result showed an indication that no stress or lymphopenia was imposed on the chicks across the treatments. Similar results were obtained by Stanton *et al.* (2008) and Akbari *et al.* (2008) who reported immune enhancement in calves and broiler chickens respectively fed organic zinc. Donmez *et al*. (2002) reported no significant differences in the WBC of broiler chicks fed zinc supplemented diets.

With increase in the dietary levels of Bioplex zinc, the heterophil component of the leukocytes decreased. Coles (1986) reported that tissue destruction irrespective of its cause and haemorrhage will result in an increase in the number of circulating heterophils it can be observed that Bioplex zinc did not elicit any tissue destruction across the treatments. The result was in agreement with report of Stanton *et al.* (2008) who observeded that organic trace minerals stimulate the immune system and enhanced eosinophils which are slightly phagocytic in calves supplemented with higher organic levels (600mg/kg Zn).

The mean values for monocytes and eosinophil were significantly lowered although no particular trend was followed, with birds on the control having the highest values. The results showed the enhancing phagocytic and parasitic roles played by the immunological responses of birds fed dietary levels of Bioplex zinc. The non significant

effect of dietary levels of Bioplex zinc on Basophils was obtained. Bioplex zinc has been reported as a non heamatotoxic mineral (Cao *et al.* 2000) and thus there was no allergy or hypersensitivity to the diets fed to the experimental diets across the treatments.

5.1.3 Serum Biochemical Parameters

The result suggests that there were no interference in the protein metabolism of the chicks. This could be inferred that the chicks did not suffer from hypoalbuminemia which can result from intestinal malabsorption, malnutrition and liver disease (Duncan *et al.,* 1994). Eggum (1976) and Tewe (1985) reported a positive linear correlation between protein quality and total serum protein concentration. The globulins are important fractions of the serum proteins that are involved in the various immunological responses (Murray *et al.,* 1988). Thus, Bioplex zinc did not alter the antigenic effect of serum globulin and the increase levels of total protein and albumin in the sera of the birds. This study suggests that protein synthesis was not impaired. According to Iyayi and Tewe (1998), serum protein and albumin synthesis are related to the amount of available protein. The pattern of Albumin/Globulin ratio followed the same trend as albumin. Duncan *et al.* (1994) reported that if albumin is selectively lost/or deficient, the albumin/globulin ratio will be low, but if there is a coconmitant loss or failure to synthesize globulin, hypoproteinemia and a normal albumin/globulin ratio may occur. The results of this study showed that there was no selective loss or deficiency in the serum albumin thereby resulting in normal albumin/globulin ratio. This result was at variance with report of Idowu *et al.* (2011) and Lavengood *et al.* (2000), however, it is in support of Aksu *et al.* (2010) because the higher the blood protein, the greater the body fortification.

The role of zinc as a co-factor in the synthesis of enzymes required in the carbohydrate and lipid metabolism was reflected in the glucose values. Zinc is associated with insulin in the pancreas, and pancreatic concentrations are markedly reduced by dietary deficiency. Zinc-deficient rats have a reduced concentration of plasma insulin and a lower pancreatic release of immunoreactive insulin (O'Dell, 1997). This indicated the

biological interaction of Bioplex zinc as insulin mimetic. The mean values were within the range recommended by Mitruka and Rawnsley (1981). This corroborates the finding of Levengood *et al.* (2000) and at variance with that of Parak and Strakova (2011) who fed BZ to cocks. The results of serum urea showed the nephron/glomerater filtration rate of the chicks were not impaired by the dietary levels of Bioplex zinc.

The creatinine values were not significantly influenced by the dietary Bioplex zinc levels. This corroborates the finding of Idowu *et al*. (2011) who did not record any significant difference in the creatinine of point of lays fed zinc proteinate. Creatinine is a non protein nitrogen substance formed during muscular metabolism of creatinine and phosphocreatinine. It is excreted by glomerular filtration. Osborne *et al*. (1972) reported that a marked elevation in serum creatinine concentration indicates severe impairment in the functioning of nephrons. It can be inferred that the birds did not suffer impairment in the functioning of their nephron. Eggum (1976) and Tewe (1985) reported a negative correlation between protein quality, serum urea and creatinine concentration.

According to Aksu *et al,* (2010), there is an association between blood levels of cholesterol and the risk of coronary heart disease in humans and premature development of artherosclerosis. It is evident form the study that Bioplex zinc was able to moderate the scouring of excess cholesterol from the artery wall participated in the regulation of cell growth, differentiation and diverse cell functions. The result was consistent with the findings of Parak and Strakova (2011) and Aksu *et al.* (2010) and contrary to the finding of Frimpong and Magee (1986) who reported a significant increase in the triglycerides of male rats fed dietary Zn up to 200mg/kg. Elevated AST due to toxic substances have been reported in chicks (Ologhobo *et al.,* 1982). Bone alkaline phosphates activity is invariably reduced in Zn deficiency but the significance of this is not clear. Since AST is an indication of tissue degeneration especially skeletal and cardial muscle, it may be inferred that the experimental chicks across the treatments did not suffer any tissue degeneration. This result supports the findings of Idowu *et al*. (2011) who reported significant differences in ALP and AST and no significant differences in ALT when fed to laying hens. Coles (1986) reported that increased serum alanine transferase (ALT) and

Alkaline phosphatase (ALP) activities are used as indicators of liver insufficiency and bone abnormalities respectively. From the result obtained in this study for the serum enzymes it may be inferred that all the experimental chicks across the treatments for ALT and ALP did not suffer any liver damage or bone abnormalities.

5.2: STUDY 2: GROWTH, HAEMATOLOGICAL, SERUM PARAMETERS, CARCASS AND RELATIVE ORGAN CHARACTERISTICS OF GROWING COCKERELS FED DIETARY LEVELS OF BIOPLEX ZINC

5.2.1 Performance characteristics of growing cockerels

The performance characteristics of growing cockerels showed that the daily feed intake, daily weight gain, final live weight and feed conversion ratio were not significantly altered by the dietary treatments. This finding is in agreement with the results of Nollet *et al.* (2008) who reported no significant effect on performance of broiler diets supplemented with Cu, Fe, Mn and Zn. during the entire trial of 42 days and in contrary to the findings of Tactacan (2001) who reported no difference in the body weight and feed conversion ratio of broilers fed proteinated supplements and Rossi *et al*. (2007) who indicated that organic Zn (Bioplex zn) does not affect growth performance of broilers but increases resistance of skin to tearing therefore improving carcass quality. The increase in weight compared to the control may therefore be attributed to the growth promoting effect of zinc through the quantitative modification of the microbial gut flora. Hedemann and Jensen (2004) reported that after weaning, it is well established that villus atrophy is observed in the pig small intestine and a decrease in the activity of digestive enzymes in the pancreatic tissue has been reported. It is speculated that supplementing pig diets with Zn and Cu may promote the processes of tissue repair in the small intestine and stimulate the synthesis of digestive enzymes, resulting in a better digestion and absorption of nutrients and potentially improving growth performance.

The feed conversion ratio (FCR) was not significantly different across the treatments. The higher the feed conversion ratio, the less desirable or efficient is a treatment. These results were consistent with a previous report which showed that supplementing a basal

diet of broiler chicks with Zn from Availa Zn, an® amino acid zinc complex, improved feed conversion without altering growth rate (Burrell *et al.*, 2004)

5.2.2 Haematological characteristics of growing cockerels fed dietary levels of Bioplex zinc.

The Packed cell volume, haemoglobin and erythrocytes observed in the birds in all the treatments were within the normal range reported by Mitruka and Rawnsley (1981) and Jain (1986). This suggests that the blood of the birds had an appreciable oxygen carrying capacity and that the treatment had no adverse effect on the health status of the animal as low PCV values are taken to indicate anaemia.The ability of the immune system to be a highly proliferating cell system strictly depends on the availability of Zn in several species (Dardenne and Bach, 1993).

The result obtained in the WBC values supports that of Sunder *et al*. (2008) and Huang *et al.* (2007) who reported an improved immune response and alleviation of stress in broiler chickens up to four weeks of age. This could be attributed to zinc essential functioning of the immune system to increase the counts of thymocytes and peripheral T cells, an activity of natural killer cells but at variance with the findings of Johnson and Fakler (1998) and Wellinghausen *et al.* (1997) who reported that higher levels of Zn beyond the physiological limits of an individual may have no beneficial effect on immune responses. Zinc, has a role in chicken immune function and resistance to diseases (Kidd *et al.*, 1996). Zinc contributes in the differentiation of many cell types including immune cells by regulation of DNA transcription, in the form of zinc finger proteins (Luscombe *et al.*, 2000). Therefore birds given a diet marginal in zinc showed a decrease in humoral immune response (Burns, 1983). However, using Zn from an inorganic source, Pimentel *et al.* (1991) has reported no improvement in immune function of chickens.

5.2.3 Serum biochemical parameters of growing cockerel fed dietary levels of Bioplex zinc.

From the result obtained for the growing cockerels, similar results with glucose, cholesterol, triglycerides and AST were obtained and reported in the previous chapter

for cockerel chicks fed dietary levels of Bioplex zinc. It has been reported that the proteolytic activity of the pancreases was found to be reduced in the Zn-deficient rat. Pancreatic carboxypeptidase was later shown to be a Zn metalloenzyme. Carboxypeptidase activity was then found to be appreciably reduced in Zn-deficeint rats and to return rapidly to normal with Zn therapy (Meftah, 1984). Higher zinc intakes were associated with lower HDL-cholesterol levels Aksu *et al.* (2010). Zinc proteins are involved in the transcription and translation of genetic material, perhaps accounting for its essentiality to all forms of life. Based on the total protein values, it can be deduced that the utilisation of amino acids in the synthesis of protein was not impaired. This could be due to the normalcy in the synthesis or degradation of ribonucleic acid or both. As zinc is associated with insulin in the pancreas, and pancreatic concentrations are markedly reduced by dietary deficiency, the report that Zinc-deficient birds have reduced concentration of plasma insulin and lower pancreatic release of immunoreactive insulin. The study indicated that the liver of the birds was not diseased nor suffered from the treatments. This finding supports the work of Parak and Strakov (2011) who reported a significant decrease in the total cholesterol and no significant difference in the AST and ALT of breeder cocks fed Bioplex zinc. However, Kucuk *et al.* (2008) did not confirm any significant changes in the concentrations of total cholesterol, triglycerides and glucose when the feed mixtures were supplemented with organic complexes of zinc to broiler chickens.

5.2.4 Carcass characteristics of growing cockerels fed dietary levels of Bioplex zinc.

The result of carcass analysis shows increase in body weights of birds on experimental diets This could be due to the presence of testosterone being enhanced by zinc which facilitated accelerated muscle growth and proper tissue development as a result of good nutrient utilization. This confirmeds the observation of Fahey (1998) that anabolic effect of androgen includes accelerated growth of muscle. Recently, more emphasis has been placed on formulating feed to achieve goals other than growth such as improved immune response or improved carcass yield. The results of a study conducted by (Rahman *et al*., 2008) showed the tendency that up to 120mg/kg of three organic (zinc acetate, zinc-

methionine and zinc lysine) zinc sources had a significant increase in breast and carcass weight percentages of broilers fed supplemental levels of organic zinc. In the studies of Teeter and Dehyhim, (1996) and Skinner *et al.* (1992) withdrawal of trace minerals from the growers diet failed to impact carcass criteria, and that can indicate that the relationship between trace minerals and carcass quality traits needs more clarity. However, Rossi *et al.* (2007) reported that weights of individual meat cuts of broilers were not significantly influenced by increasing organic Zn levels in the diet.

5.2.5 Relative weights of some organs of growing cockerels fed dietary levels of Bioplex zinc

The dietary levels of Bioplex zinc fed to experimental cocks were all significantly similar in the values of the relative weights of liver, heart, lung, spleen, gastro intestinal tract (GIT), testes, gizzard and pancrease except kidney. Although the value of the kidney was significantly different, the numerical variations across the treatments were not consistent. Kidney appeared to be more sensitive as an organ because 10% of the experimental cocks fed 300 mg/kg were characterized by renal congestions of the blood vessels. Other organs appeared to be more stable to dietary Bioplex zinc up to 300mg/kg.

This finding did not agree with Rahman *et al*. (2008) who reported a significant increase in liver of broilers fed supplemental levels of organic zinc, Lavengood *et al.* (2000) reported decreased body, liver, pancreas, gonad, and gizzard weights; with increased kidney weight of ducks fed organic zinc, Sunder *et al. (*2008) reported the weight of spleen and bursa of broiler chicks fed supplemental zinc diet was significantly influenced while Akbari *et al*. (2008) reported an increase in the weights of spleen of broiler chickens fed supplemented zinc. Tactacan (2001) suggested that pancreas was the most sensitive soft tissue to dietary Zn for chicks. From this study, the effect of Bioplex zinc on the kidney did not follow any particular trend, therefore one cannot

make a categorical statement about what could have triggered this difference in size hence further investigation may be carried out to buttress this fact.

5.2.6 Organoleptic characteristics of growing cockerels' meat as influenced by dietary levels of Bioplex zinc.

Meat colour is the first criterion that consumers' use to judge meat quality and acceptability (Comfort. 1994), while the appearance of meat influences the consumer's acceptance of the meat (Van Oeckel *et al.*, 1999). From the taste panelist score, the aroma, colour, flavor and tenderness were not adversely affected by the treatments. The panelist results showed that birds on treatment 1 had the poorest juiceness rating. This may be attributed to the low level of intramuscular lipid present in cut. Juiceness is directly related to the intramuscular lipid and moisture content of the meat (Cross *et al.*, 1986). This finding was at variance with the claim of Hafez (1993) that androgenic compound improve the carcass quality by decreasing the amount of fat and increasing the proportion of edible tissue.

5.3. STUDY 3: PERFORMANCE, NUTRIENT DIGESTIBILITY, ZINC RETENTION AND HISTOPATHOLOGICAL EXAMINATION OF COCKS FED DIETARY LEVELS OF BIOPLEX ZINC

5.3.1 Performance Characteristics of Cocks

There was a significant difference in the feed intake during the cocks' phase of the experimental birds, thus dietary Bioplex zinc could be responsible for the higher weight gain and the supplemental diets coupled with the quantitative modification of microbial gut flora present in the gut. According to Webb *et al.* (2005) who reported that organic minerals that are chelated to small peptides have much greater mucin layer and results in lower competition between bioavailability through increased selective transport of minerals at gut level. This has a positive effect on growth performance.

This result obtained in this study also agreed with Cao *et al.* (2000), Ao *et al* (2006) who reported that feed intake and daily gain were greatest in birds supplemented with organic Zn compared with those supplemented with inorganic Zn but in variance with the

findings of Mohanna and Nys (1999) who reported that weight gain, feed intake and feed conversion ratio in broilers were not influenced by Zn sulfate or Zn-Methionine. The improvement in weight gain and the gain per feed ratio observed for experimental birds fed Bioplex zinc supplemented diets in this study however contrasted the report of Ao *et al.* (2006), Tactacan (2001) and Sunder *et al.* (2008) who reported that inclusion of zinc in the diet of broiler growers had no effect on weight gain, feed intake and feed efficiencies. However, the report(s) is in variance to the work of Osineye (2009) unpublished who reported that goat fed dietary zinc beyond 164.0mg/kg recorded a higher feed conversior ratio value of 10.67 while Nollet *et al*. (2007) reported there were no differences in performance between the birds fed the high inorganic minerals and the broiler chickens fed the low organic chelates. This could have been facilitated by the presence of testosterone which plays an anabolic role since zinc enhances the production of it. This support the claims of Hafez (1993) that testosterone enhance growth and increased protein anabolism.

The enhancement of wound healing by Zn to enhance growth may stem from a heightened metabolic demand for this element for collagen synthesis in the process of tissue repair, with an increase in collagen synthesis and cross-linking explaining gains in wound tensile strength but direct evidence for this is lacking. Furthermore, Zn responsive differences in tissue repair could be related to differences in the rate of cell division and DNA production in rapidly regenerating tissue. It has since been have shown that depressed activity of thymidine kinase for DNA synthesis and cell division is an early metabolic defect on Zn deficiency (McClain *et al.*, 1973; Ibs and Rink, 2003). This study has demonstrated that the supplementation of cocks' diet with Bioplex zinc has a pronounced increase on their weight gain and feed conversion efficiencies.

The coefficient of determination R^2 values for performance traits can be used to predict the performance of the cocks fed dietary levels of BZ. The R^2 values for daily feed intake, daily weight gain and final body weight were significant and their respective prediction equation will give a strong and more reliable prediction of these parameters.

Based on the scope of this study up to the 300mg/kg BZ inclusion levels, a progressive linear regression of the daily feed intake was predicted (Figure 7), thus an optimal level of performance for BZ could not be determined. A polynomial regression of the weight gain (Figure 8) predicted the equations above respectively. In poultry, a wide tolerance range of 800-3000mg/kg occurs between the physiologic and toxic levels (Larry, 1987) while NRC (1980) gave a mineral tolerance level of 1,000ppm to swine, turkeys and chickens fed nonpurified diets. To this effect, further research studies of increased dietary levels beyond 300mg/kg are needed to ascertain an optimal level of performance for cocks fed BZ

5.3.2 Haematological responses of Cocks

The haematology results of the experimental cocks revealed that the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH); white blood cells and heterophils were significantly influenced by the dietary Bioplex zinc.

Babatunde *et al.* (1987) stated blood is an important index of the physiological, pathological and nutritional status of an animal. Although the values of MCV and MCH were significantly decreased, they did not follow a particular trend, but they were within the physiological range of a normal chicken recommended by Mitruka and Rawnsley (1981). In line with this report, the experimental birds did not exhibit any form of anemia as their physical conditions did not shown sign of inferior health status, as the functions of the haemoglobin were not impeded by the dietary Bioplex zinc.

The significantly decreased levels of circulating white blood cells showed the immunological defence ability of Bioplex zinc in the diets. Leukocytosis which is the elevated level of blood leukocytes was not reported thus it can safely be inferred that dietary Bioplex zinc did not provoke immunologic disorder in the cocks across the treatments. Dielder (1998) reported that heterophils form the first line of defence against

infection and plays important roles in phagocytosis, lymphocyte activation and cytotoxicity. The significantly lower values of heterophils obtained for cocks on treatments 2, 3 and 4 compared to the control, coupled with the lowered levels of leukocytes in the cocks is an indication that dietary Bioplex zinc was not toxic to the body cells (cytotoxicity of the cocks).

This finding is in support of Hudson *et al.* (2004) who indicated that broiler breeder hens provided diets supplemented with zinc from ZnAA (Zn amino acid chelate) might have increased thymulin activity; therefore, enhancing immune responses through increased maturation of T lymphocytes and activation of B lymphocytes by T-helper cells and the works of Sunder *et al*. (2008), Akbari *et al.* (2008) and (Kidd *et al.* 1996) who reported enhanced immune response in broiler chicks fed higher levels of zinc supplementation but contrary to the findings of Wellinghausen *et al.* (1997) who reported that higher levels of Zn beyond the physiological limits of an individual may have no beneficial effect on immune responses.

Nevertheless, Zn plays an important role in immunomodulation by increasing the counts of thymocytes and peripheral Tcells and also by enhancing the production of interferon (Kidd *et al.* 1996), which might have been elevated by the dietary increases of Zn in this study. The normal values for AST and ALT were indicative of normal functionings of the liver of the experimental birds as extensive tissue destruction of these enzymes are liberated into the serum thereby leading to higher levels in diseased states as manifested in the sera of patients with acute hepatic disease (Cornelius, 1970)

5.3.3 Serum biochemical parameters of experimental cocks

The dietary levels of Bioplex zinc on cocks revealed that the values of glucose, cholesterol and triglycerides in the experimental cocks were significantly lowered as the level of Bioplex zinc increased in the diet.

Zinc is necessary for the proper functioning of many enzymatic systems, and the insulin system is probably the most important one. It also plays a significant role in various peptidases, esterases and dehydrogenases. It influences the immune system, DNA

synthesis, cell proliferation, protein synthesis and the incorporation of iron into the haemoglobin (Parak and Strakov, 2011). The above results are supported by data from many researches in the area of veterinary medicine. The anti-atherogenic effect of zinc in hypercholesterolemic rabbits was reported (Ren *et al*. 2006). Zinc deficiency caused increased plasma lipid levels and an increased risk of cardiovascular diseases in LDL receptor knock-out mice. Mice with zinc-deficient diets exhibited increased cholesterol and triglycerides levels in blood plasma (Reiterer *et al*. 2005). Bolkent *et al*. (2006) proved the protective effect of zinc supplementation on lipid metabolism indices (total lipids, cholesterol, HDL-cholesterol) in laboratory rats with streptozotocin-induced type 1 diabete.

5.3.4 Organs histopathology

From the study, since the AST, urea and creatinine values in the sera of the experimental cocks were not significantly different and within the physiological range reported by Mitruka and Rawnsley (1981) and their principal indicators occur in the liver and kidney organs. It may explain why there were no hepatotoxic effects of the Bioplex zinc in the liver, no induced cellular proliferation or enlargement of the cells of the heart, lung, spleen and pancrease and no observable lesions or degeneration of the seminiferous epithelium in the testes of the cocks across the treatments. However the renal histological congestions of the blood vessels observed in the kidney of 10% of the cocks fed and sacrified at 300mg/kg may not be linked to the zinc action; nevertheless further investigation should be carried out to validate these results.

The result of this study supports the finding of Wegger and Palludan (1978) who reported that boars that were Zn deficient had reduced testicular weights and their histological examination revealed abnormal structure of testes. Also in line with those of Levengood *et al.,* (2000) who reported mild to moderate necrosis of the epithelial cells of the renal tubules displayed by the ducks that were examined histologically in their study. However the result obtained in this study revealed that dietary levels of Bioplex zinc beyond 200mg/kg may adversely affect organs histopathology.

5.3.5 Apparent Nutrient Digestibility

According to Ammerman *et al.* (1995), bioavailability is defined as the degree to which an ingested nutrient is absorbed in a form that can be utilised in metabolism by the normal animal. This definition stresses that the mineral must be available not only at the dietary level but also at the tissue level. Several factors may influence the bioavailability studies, such as the assessed indicators of mineral status (eg measurement of liver Cu or bone Zn, alkaline phosphatase activity), the mineral status of the animals and the composition of the experimental diet, including the level of trace mineral supply (Jondreville and Revy, 2003).

The apparent digestibility of dry matter from samples collected was not influenced by the levels of Zn in the diet. This may suggest less interference at the gut absorption sites. Ash is an indication of mineral nutrients. The significant higher values of ash and crude protein of cocks fed the test ingredient could be attributed to the fact that Bioplex zinc is a source of zinc trace mineral element. These results agree with Underwood and Suttle (1999) and Leeson (2005) who reported the protolytic role of zinc in metabolic processes which must have also occurred in this study. Hence zinc has enhanced the digestibility of the nutrient by the birds. The levels of zn-retention may be as a result of the high digestibility of nutrients in the diets of birds especially on zinc level inclusions thus the potentiality of the diets to enhance Zn- utilization. The positive Zn- balance recorded in this study is evidenced in the birds gaining weight during the period of experimentation.This therefore suggests that the different zinc level inclusions could be deemed adequate in fortification

5.3.6 Zinc utilization

From this study, the zinc retention (% of intake) mean values were significantly influenced by the dietary levels of Bioplex zinc and increased across the treatments. This finding is in agreement with Thomas and Ravidan (2010) who reported performance was maintained in ducks despite reduced mineral intake, indicating that Bioplex organic trace mineral are more available and better utilized by the ducks. Nollet *et al.* (2007) and Sunder *et al.* (2008) have reported better Zn utilization and retention in broiler chicks

fed supplemental zinc diets. Studies with swine tend to show similar results as those found in broilers. Coffey *et al.* (1994) observed improvements in piglet performance when copper lysine was used as compared with copper sulfate. On the other hand, Apgar *et al*. (1994) did not verify any response of this combination in growth. Hill *et al.* (1986) found higher feed intake with the use of zinc methionine, but feed conversion ratio was not influenced when that chelate was combined with copper sulfate. Higher nominal zinc absorption was found by Hill *et al*. (1986) for zinc-methionine as compared to zinc chloride, suggesting differences in transport rates between these two sources.

Animals fed chelated sources of zinc have been reported to excrete lower amounts in their faeces and so there is less environmental contamination (Steveson, 2009). Opinion of the Scientific Committee for Animal Nutrition Europe SCAN (2003) on the use of zinc in feedingstuffs reported that inorganic minerals may form insoluble complexes with other dietary agents resulting in low absorption. Also the poor retention and high excretion rates of inorganic minerals led to environmental concerns during the 1980s and 1990s. The European Union is concerned about possible detrimental effects of excess supplementation with trace minerals on the environment or human and animal health. Fortunately, research in trace element nutrition has led to the development of more bioavailable organic minerals, including trace minerals derived from chelates. Chelates allow a lower supplementation rate of trace minerals with an equivalent or improved effect on animal health, growth and productivity.

This result suppors the work of Carlson *et al.* (2004) who reported gain nutrient digestibility and Zn utilization without loss of growth performance in weaned piglets fed supplemental zinc diets. While Nollet *et al*. (2007) and Peric *et al*. (2007) reported no differences in performance between the broiler chickens fed the high inorganic minerals and the birds fed the low organic chelates, the authors concluded that the use of organic trace minerals permits a reduction of at least 33% in supplement rates in comparison with inorganic minerals, without compromising performance.

In weaned piglets, Carlson *et al.* (2004) evaluated various supplementation rates of organic Zn in the form of a proteinate (Bioplex Zn, Alltech) or as a polysaccharide complex (Sea-Questra Min Zn, Quali Tech), and compared these with ZnO (Zinc oxide) at 2,000mg/kg. Feeding lower concentrations of organic Zn greatly decreased the amount of Zn excreted in comparison with inorganic Zn, without loss of growth performance. Stevenson (2009) reported in the series of trials on faecal mineral excretion from birds fed Bioplex zinc compared to inorganic minerals that it is possible to feed lower levels of minerals in an organic form and maintain the performance of ducks while reducing the levels of minerals excreted in the faeces.

Nollet *et al.* (2007) reported that mineral excretion was higher in manure of birds fed at 2/3 and 3/3 levels of recommendation in organic mineral than in those of birds fed at 1/3 levels of organic minerals. It is presumed that the body becomes "loaded" with these minerals. Organically complexed minerals can be easily absorbed by intestines in comparison to inorganic salts but not be completely utilized in high levels.This finding was supported by (Bao *et al.* 2007; Burrell *et al.* 2007). Similarly, Nollet *et al.* (2008), indicated that the mineral excretion of birds fed at 100% and 67% portion of organic mineral premix were not significantly different from the birds fed at 100% levels of recommended levels in inorganic minerals. Tactacan (2001) and Leeson (2003) reported that organically complexed trace minerals provide alternative pathways for absorption, thus leading to a reduction in the excretion of minerals. Osineye (2009) reported Zn retention values of 29.9 to 40.6% by West African Dwarf kids fed varying levels of zinc. This values fall within the higher range (34.9-69.1%) obtained in this study. Proper zinc utilization when dietaty zinc was adequate to synthesis enzymes and proteins which are necessary for optimal growth, reproduction, development with less environmental pollution could be inferred.

5.3.7 ENVIRONMENTAL IMPACT

Overall, the present findings showed that the retention of zinc minerals in cockerels is high. This also has implications for the excretion of minerals into the environment, which is becoming a worldwide issue. A potential advantage of chelated trace minerals

is the high retention which provides opportunities as trace mineral chelates in dietary manipulation to improve utilisation of minerals. Compared to inorganic sources, chelates of Zn, Cu and Mn have been shown to confer stability in the upper digestive tract, thereby avoiding binding with antagonists and allowing the complex to be delivered to the absorptive epithelium of the small intestine for mineral uptake and improving bioavailability (Leeson, 2005; Bao *et al,* 2009). The use of organic sources may provide even greater mineral retention allowing the requirements of birds to be met with lower mineral levels of inclusion in the diets (Leeson and Summers, 2001)

5.4. STUDY 4: SEMEN CHARACTERISTICS OF COCKS FED DIETARY LEVELS OF BIOPLEX ZINC

5.4.1 Age at puberty

The effect of age of cocks on semen physical characteristics is very important in the poultry industry. Attaintment of the pubertal age of the cocks was determined by the presence of mature spermatozoa in the exudate obtained from the distal end of the papillae within the cloaca. Cocks fed diets on treatment 4 attained sexual maturities on the 126 day, three, five and ten days ahead of cocks on treatments 3, 2 and 1 respectively. The early attainment of puberty particularly by cocks on treatment 4 could be due to the possible enhancing effects of Bioplex zinc on the reproductive performance in cocks to enhance spermatocytogenesis. There is paucity of information in literature on attainment of cocks fed zinc sources.

5.4.2 Semen Characteristics

Semen characteristics and assessment are important indicators of the reproductive potential of breeding cock (Ajayi *et al*. 2011). The reproductive potential of poultry birds (cocks) is determined to large extent by the quality of the semen it produces (Prasad, 1993). The semen volume values obtained for the birds in this study were considerably lower than the 0.34 mL and higher than the 0.25 mL obtained by Nganga (1989) for exotic and local breeds of cock respectively, also lower than the 0.58 and 0.80m reported by Nwagu *et al.* (1996) for white and Red Rhode Island cocks respectively and also lower than the range reported (0.37-0.73 mL) for Nigerian

indigenous breeds by Peters *et al*. (2008). However, the volume of semen obtained in this study fell within the range of 0.1 to 1.0 ml reported by Sturkie (1970) and (0.10–0.33 mL) by Malik *et al,* (2013) for other adult cocks which is consistent with this study. The values of the semen volume were not influenced by the significant effects of dietary treatments on the body weights of the cocks as observed by Malik *et al,* (2013). LeDec *et al.* (1981) reported that the amount of semen volume obtained may be occasioned by the method of semen collection, age and amount of seminal fluid in the semen.

The dietary treatments significantly increased sperm motility across the treatments. The values recorded for motility in this study were higher to 64.0 % motility reported by LeDec *et al*. (1981), 37.1 % reported by Nwakalor *et al, (*1986) and 79.26 % and 72.68 % reported by Nganga (1989) for local and exotic cocks and 57.1% by Malik *et al.* (2013). This may be due to breed differences and the age of the birds. The results obtained showed that progressive motility increased with increased dietary levels of Bioplex zinc. It will be expected that the presence of zinc in the sperm tail assists in the propelling of the flagellum. The nutritive substances for sperm motility and viability in the seminal fluid might have not been depressed by dietary Bioplex zinc. Zinpro (2002) and Prasad (1993) reported that feeding Zinc to male breeders chicken increased the sperm concentration and sperm motility. White (1993) showed that zinc-deficiency induced anorexia and caused reduced secretion of gonadotrophin-releasing hormone from the hypothalamus of ram lambs and retarded testicular growth. This will lead to impaired fertility in the ram. Reduced reproductive performance has been observed in both male and female dairy cattles fed zinc deficient diets (Wilde, 2006).

The sperm concentration values were significantly increased across the treatments which suggest that all the cocks were potentially fertile as the sperm concentration was more than the minimum number of sperm 25million (De, Reviers and Willians, 1981) required for optimum fertility in natural mating. For artificial insemination programme, and by the recommendation of 25 million spermatozoa for high level of fertility in chickens, sperm concentrations obtained from this study would successfully inseminate 12 to 15

hens, all things being equal for motility and volume. The results also give an indication that spermiogenesis was not impaired across the treatments. Since sperm output is a function of semen volume and sperm concentration, the same explanation holds. Cocks on treatment 4 had the heaviest body weight with the largest sperm concentration. This may suggest that body weight may be a major factor in measuring fertility in cocks. These results are in support of the findings of Nganga (1989) and Hartoma *et al.* (1977) who reported increase in sperm counts in male subjects with oligospermia when fed with zinc supplemented diets.

The percent live sperm cells were significantly increased by the dietary levels of Bioplex zinc across the treatments. This supports the reports of Malik *et al*. (2013) who obtained 97.5% in domestic cocks. The study suggests Bioplex zinc probably did not exhibit any spermaticidal effects. This reason may further explain the result of a significant increase in the spermatozoa motility and concentration across the treatments. Thus, the spermatogenesis and semen quality characteristics of cocks fed dietary Bioplex zinc were not adversely affected.

Malik *et al*. (2013) reported a pH of 7.0–7.4, Gebriel *et al*. (2009) a pH of 7.27 and Lake and Stewart (1978) reported that the pH for semen maximal motility is 7.4 while for retention of fertilizing capacity is slightly less pH 7.1. Ajayi *et al*. (2011) reported 7.03 for Nigeria indigenous cocks. These are in variance with the lower values of 6.52–6.67 obtained in this present study. Acidic ejaculate (lower pH value) may indicate one or both of the seminal vesicles are blocked. A basic ejaculate (higher pH value) may indicate an infection. A pH value outside of the normal range is harmful to sperm. (Semen Analysis Wikipedia, 2007).

The most obvious evaluation of semen quality is colour (Peter *et al*, 2008). The results of semen colour as affected by strain could indicate that the further away from creamywhite colour the semen of the chicken strain is, the more likely is the presence of contaminations (Etches, 1998).The colour of ejaculates was consistent for all the cocks and fell within the normal colour range reported by Sturkie (1970). The semen

consistency which is colour dependent and mass activity was consistent for the birds across the treatments.

Spermatogenesis and the development of the primary and secondary sex organs in the male and all phases of the reproductive process in the female can be adversely affected by Zn deficiency. The major abnormality in the male is testicular hypofunction affecting both spermatogenesis and the production of testosterone by the Leydig cells (Underwood, 1981). Egbunike (1994) reported that the meiotic transformation of primary spermatocytes to secondary spermatocytes and the maturation of spermatids and spermatozoa require testosterone whose production is stimulated by Leutinizing Hormones thus the availability of sufficient Zn for incorporation of high amounts into sperm during the final stage of maturation is essential for the maintenance of spermatogenesis and survival of the germinal epithelium.

It can be concluded that fortification of cocks diet with zinc improved semen characteristic such as ejaculate volume, progressive motility, sperm concentration, live spermatozoa, total sperm ejaculate and motile sperm ejaculate with reduced sperm abnormalities compared with those fed diets unfortified with dietary zinc.

5.4.3 Sperm morphology

Morphology is a predictor of success in fertilizing oocytes during in vitro fertilization. Up to 10 % of all spermatozoa have observable defects and as such are disadvantaged in terms of fertilising an oocyte (Semen Analysis Wikipedia, 2007). The low percentage of sperm abnormality of less than 10% observed in the semen of the cocks is insignificant as to cause fertility worries on cocks (Ajayi *et al*. 2011). Morphological sperm cell defects that were significant in this study were Rudimentary tails (RT) and Curved midpiece (CMP)). Cocks on treatment 2 had the highest value of RT (2.44 %), followed by CMP highest value of 2.26 % for cocks on treatment 1, Maximum allowed frequency of sperm head, midpiece and tail abnormalities have also been put at 20 %, 5 % and 5 % respectively (Laing, 1979). The figures obtained in this study didn't exceed the percentages which could have resulted in reduced fertility.

lmproper handling of ejaculates during processing for microscopy has been identified as a major cause for sperm abnormality. Ejaculation of sperm with high proportion of morphologically abnormal spermatozoa, lowered fertility and interference with spermatocyte/ spermatid maturation has also been reported by Egbunike *et al*. (2007) and Wettemann and Desjardins, 1979). Usually sperms abnormalities indicate disturbances of spermatogenesis and this could be attributed to age, nutrition and pollution (Bah *et al.*, 2001). From this study, heavy body weight (2.56kg) cocks had significantly higher values of sperm concentration, live sperms, sperm motility and significantly lower abnormal sperms than light and control (2.40kg) body weight cocks. This findings support that of Gebriel *et al*. (2004). In this study, cocks on treatments with Bioplex zinc had increased number of sperm cells, reduced number of sperm abnormalities and enhanced spermatogenesis. These positive attributes could be due to the combined androgenic activity and anabolic properties of Bioplex zinc® to enhance spermatogenesis without inhibiting gonadotropin secretion.

5.4.4 Testicular Morphometry

The growth and development of the testes in farm animals have been well documented by many workers (Rekwot *et al.*, 1987; Etches, 1996). Testicular development in domestic animals has also been related to their short and long-term reproductive function (Romero-Sanchez *et al.*, 2007) which is reported to be under genetic control (Barbato, 1999). Dietary zinc deprivation has been reported to impaired gonadal growth in sexually immature rats with least testicular weights when compared with pair-fed control rats (McClain *et al.* 1984).

From the results, there were no significant effects of the dietary Bioplex zinc in all the testicular and epididymal characteristics. The paired testes weight obtained in this study were within the range of 14 and 60 grammes reported by Hafez (1993). Though cocks on treatment 4 had the numerical higher body weight, than other experimental cocks this did not influence or reflect on the sizes of those testes. The mean weight of the right and left testes across the treatments were not significantly different and is consistent to the findings of Clulow and Jones (1982). From the study, the left testes were observed to the

heavier than the right testes across the treatments. This is in agreement with the reports by Nganga (1989) and in variance with the finding of Obidi *et al.* (2008) who reported that the paired mean testicular weight value of 11.60±0.5 g for Shikabrown cocks. The paired mean testes weight reported by Adeyemo *et al.* (2007) as 5.72±2.43g for Isa brown cocks was significantly lower to the range (25.20±0.3-28.10±0.7 g) of the paired mean testicular weights obtained in this study. These differences may be due to the ages at which this parameter were determined and by genetic factors. However the apparent superiority of the left testes over the right testes reflected on their sperm production and storage potentials as there could be differences in spermatogenic capacity between the right and left testes in breeder cocks.

Epididymal and tunica albuginea weights also showed no significant treatments and were a reflection of the weight of the testis. For the study the left traits appear superior to the right for all the testicular and epididymal characteristics considered. Similar observations had been made by Nganga (1989). This probably explains why the rate of spermatogenic activity taking place in the left organs in higher than in the right organ. The result obtained in this study showed that body, testes and epididymal weights were not significantly affected by the dietary Bioplex zinc.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY

The potential of Bioplex zinc, a chelated proteinate available for poultry feed supplementation to meet the higher nutritional needs for rapid growth, reproductive performance and animal health were evaluated with a view to assess its effect on growth, organ histopathology and semen characteristics of cockerels fed dietary levels of an organic zinc source.

Four dietary treatments were prepared to contain 0, 100, 200 and 300mg/kg Bioplex zinc for diets 1, 2, 3 and 4 respectively.

240 day-old Nera Black cockerel chicks were allotted randomly to the experimental diets at the starter phase of 8 weeks of feeding trial. The performance indices of the chicks such as daily weight gain and final body weight were significantly increased by the dietary levels of Bioplex zinc. Some heamatological parameters namely, mean corpuscular volume, mean corpuscular haemoglobin and white blood cells were significantly altered as the Bioplex zinc levels increased. The values of some serum biochemical parameters namely the glucose, cholesterol, triglycerides and aspartate aminotransferase were significantly decreased up to the 300mg/kg inclusion levels of Bioplex zinc and throughout the physiological phases of the chicks thereby indicating Bioplex zinc as a non haematotoxic and non immunotoxic substances.

The same sets of birds used in experiment one were further investigated in a ten week study for the growers phase. The growth performance, haematology, serum biochemical, carcass, organoleptic histopathological, apparent digestibility and zinc utilisation

parameters were determined. The apparent digestibility and utilization of zinc were significantly influenced by the Bioplex zinc levels. The relative mean values for the liver, heart, lungs, spleen, GIT, left and right testes, full and empty gizzard and pancreas of the growing cockerels fed dietary levels of Bioplex zinc were not significantly different among the treatments except the kidney. Renal congestions were detected in the kidney of 10% of the cocks fed and sacrificed beyond 200mg/kg of Bioplex zinc inclusion levels. Spleen, liver and testes did not show any visible lesions.

The finishers phase was conducted to assess the semen characteristics of cocks fed dietary levels of Bioplex zinc.The experiment lasted 12 weeks. Attainment at puberty, sperm motility, percentage sperm live cells and sperm concentration were significantly increased as the Bioplex zinc levels increased up to 300mg/kg thereby enhancing sperm survivability. The testicular and epididymal characteristics of the birds were not significantly different across the treatments.

6.2 CONCLUSIONS

Based on the findings in this study,

- Dietary Bioplex zinc generally improved the feed intake, weight gain, feed conversion ratio and nutrient utilization across the chicks physiological phases as the dietary levels of Bioplex zinc increased.
- The haematological and serum biochemical parameters showed no detrimental effects.
- Dietary Bioplex zinc at a concentration of about 300mg/kg for a 30 week period has the potential of enhancing growth, nutrient digestibility, immune responses and sperm survivability, therefore cocks intended for breeding can be exposed to dietary Bioplex zinc of 100-300mg/kg for optimum reproductive performance without any negative effects.

- Bioplex zinc did not pose any potential health threat to the birds fed up to the maximum concentration, while cocks meant for histopathological examinations should not be exposed to Bioplex zinc in excess of 200mg/kg.

- For the serum enzymes it may be inferred that all the experimental chicks across the treatments for ALT and ALP did not suffer any liver damage or bone abnormalities.

- Role of zinc as a co-factor in the synthesis of enzymes required in the carbohydrate and lipid metabolism. Zinc is associated with insulin in the pancreas, and pancreatic concentrations are markedly reduced by dietary deficiency.

- Presence of testosterone being enhanced by zinc which facilitated accelerated muscle growth and proper tissue development as a result of good nutrient utilization.

- The mean values for the packed cell volume, haemoglobin, erythrocytes, MCHC, lymphocytes, monocytes, eosinophils, and basophils of the experimental cocks fed dietary levels of Bioplex zinc were not significantly different across the four treatments. This may suggests that the blood of the birds had an appreciable oxygen carrying capacity.

- Zinc is a co-factor in the production of lymphocytes which are precursors for thymocyte cells (Tcells) essential in immunological processes.

- Bioplex zinc was able to moderate the scouring of excess cholesterol from the artery wall participated in the regulation of cell growth, differentiation and diverse cell functions

- Since AST is an indication of tissue degeneration especially skeletal and cardial muscle, it may be inferred that the experimental chicks across the treatments did not suffer any tissue degeneration.

- Kidney appeared to be more sensitive as an organ because 10% of the experimental cocks fed up to 300mg/kg and sacrified were characterized by renal

congestions. Other organs like spleen, liver and testes appeared to be more stable to dietary Bioplex zinc up to 300mg/kg.

- Dietary levels of Bioplex zinc beyond 200mg/kg may adversely affect organ histopathology
- Fortification of cocks diet with zinc improved semen characteristic such as ejaculate volume, progressive motility, sperm concentration, live spermatozoa, total sperm ejaculate and motile sperm ejaculate with reduced sperm abnormalities compared with those fed diets unfortified with dietary zinc

6.3 RECOMMENDATIONS

The inclusion levels of Bioplex zinc up to 300mg/kg in the diets of cockerel chicks, growers and cocks will still provide a safe limit on haematological, serum biochemical parameters, organs characteristics, nutrient digestibility and the reproductive potentials of chickens.

REFERENCES

A.O.A.C (1999).Official methods of analysis of association of official analytical Chemists 15th ed. A.O.A.C. Inc. Arlington, Virginla, U.S.A.

Abubakar, A. (1997). Effects of Supplemental yeast culture on the Utilization of high dietary Inclusion of Palm Kernel meal by Pullet Chicks. M. Sc. Project, Animal Science Department. University of Ibadan, Oyo State.

Abdujalleel, S.A. and Shuhaim-Othman, M., (2011). Metal concentration in Eggs of Domestic avian and Estimation of Health Risk from Egg Consumption. *Journal of Biological Science* D0110; 3923

Acton, J. C., Breyer, L. and Sallerlee, L. D. (1982). Effects of dietary fibre constituents on the vitro digestibility of casein. *Journal of Food Science* 47: 556.

Adebowale, B. S., Olayinka, A. O., Matthew, O. O. and Oluwaseun, D. O (2009). Spermatozoa morphology and characteristics of male Wistar rats administered with ethanol extract of Lagenaria Breviflora Roberts. *African Journal of Biochemistry* 8 (7): 1170-1175.

Adeyemo, G. O., Longe, O. G. and. Adejumo, D. O (2007). The reproductive performance of breeder cocks fed cotton seed cake-based diets. *Internatonal Journal of Poultry Science* 6:140-144.

Aduku, A. O. and Olukosi, J. O. (1991). Animal Products, Processing and Handling in the Living Books series, Abuja. *G. U. publication*s. 29: 135-136.

Akinwumi, J. A., Adeleye, A. D., Ikpi, A. E., and Oyayide, S. O. (1979). Economic Analysis of Nigerian Poultry Industry. Federal Livestock Department, Lagos. pp 25.

Akbari, M. R., Kermanshahi, H., Nassiri, H., Moghaddam, A. R., Heravi Moussavi and. Tavakkol Afshari, J. (2008). Effects of Wheat-Soybean Meal Based Diet Supplementation with Vitamin A, Vitamin E and Zinc on Blood Cells, Organ Weights and Humoral Immune Response in Broiler Chickens. *Journal of Animal and Veterinary Advances* 7 (3): 297- 304.

Aksu S. D., Aksu T. and Özsoy, B. (2010). The Effects of Lower Supplementation Levels of Organically Complexed Minerals (Zinc, Copper and Manganese) Versus Inorganic Forms on Hematological and Biochemical Parameters in Broilers Kafkas Univ *Veterinary Fak Derg Research Article* 16 (4): 553-559.

Alaku, (1997). Seasonal and annual variation in body weight and carcass variation in the Sudan Savana zone of Nigeria, *Nigerian Journal of Animal Production* 24 (2): 180-187

Aletor, V.A. and Egberongbe, O. (1992). Feeding differently processed soyabean Part 2. An assessment of haematological responses in the chicken diet. Nahning: 36(4): 364-369.

Altman, R. B. (1979) Avian clinical pathology, radiology, parasitic and infectious disease. Proceedings of *American Animal Hospital Assocociation*. South Bend IN, 1979.

Ambula, M. (2014) Facilitates IC Handbook Preparation Workshop A Comparative Analysis of Transaction Costs in Collective and Individual Marketing of IC in Kakamega County, Kenya »

Amir, D., Gacitria, H., Ron, M. and Lehrer, A. R. (1986). Seasonal characteristics and the fertility of Finn Cross Rams subjected to frequent ejaculation. *Animal Reproduction of Science* 10: 75-84.

Ammerman, C. B., Baker D. B., and Lewis, A. J. (1995). Bioavailability of Nutrients for Animals. Academic Press, NewYork, pp 367-398.

Anke, M. J., Groppel, B., Krause, U., Angelow, L. Kosla, T., Regius, A. Masaoka, I. J. and Siegert, E. (1988). In "Trace Element Analytical Chemistry in Medicine and Biology" (P. Bratter and P. Schramel, eds.. Walter de Gruster, New York. 5: 607

Angel, R., Tamim, N. M., Applegate, J. T., Dhandu A. S., and Ellestad, L. E. (2002). Phytic acid chemistry: influence on phytinphosphorus availability and phytase efficacy. *Journal of Applied Poultry Research*; 11:471-480.

Aniket, R. (2005). Medline plus medical encyclopedia hyperhidiosis. Total protein division for Endocrinology and Metabolism. John Hopkins University School of Medicine, Baltimore M.d. www.wm.nih.gov/medlineplus/ency/article/ 007259.htm.

Ao, T., Pierce, J. L., Power, R., Dawson, K. A., Pescatore, A. J., Cantor, A. H., and Ford, M. J. (2006). Evaluation of Bioplex Zn® as an organic zinc source for chicks. *International Journal of Poultry Science* 5: 808-811.

Ao, T., Pierce, T., Paul, M., Brennan, K.M. and Pescatore, A. (2012). Relative bioavailability value of zinc proteinate (Bioplex Zn®) compared with zinc methionine for chicks. (Submitted to International Poultry Scientific Forum Abstracts, Atlanta, GA USA)

Apgar, G. A., Komegay, E. T. and Linderman, M. D. (1994). Effect of copper lysine chelate and copper sulfate as growth promoters for weaning swine. *Journal of Animal Science:* 72 (Suppl1): 273

Arinola, O. G (2008). Essential trace elements and metal binding proteins in Nigerian consumers of alcoholic beverages. *Pakistan Jounal of Nutrition* 7(6): 763-765.

Ashmead, H. D. (1993). Comparative intestinal absorption and subsequent metabolism of metal amino acid chelates and inorganic metal salts. In: The Roles of Amino Acid Chelates in Animal Nutrition, ed. H.D. Ashmea, New Jersey, USA: Noyes Publishers. pp 306-319.

Auckland, J. N. and Fulton, R. B. (1973). The use of low protein diets in the 12-18 weeks periods of light hybrid pullets. *British Poultry of Scienc* 14: 49-54.

Awotwi, E. K. (1990). A study of base line haematologic.al values of domestic and commercial chicken in Ghana. *Bullentin Animal Health Production of Africa*. 38:453-458.

Ajayi, F. O., Agaviezor, B. O. and Ajuogu, P. K. (2011). Semen Characteristics of Three Strains of Local Cocks in the Humid Tropical Environment of Nigeria *International Journal of Animal Veterinary Advances* 3(3): 125-127.

Babatunde, G. M., and Fetuga, B. L. (1976). Determination of minimum crude protein requirement of broiler starter and finisher in the Tropics. *Nigerian Journal of Animal. production* 3: 126-128.

Babatunde, G. M., Pond, W. O., Krook, L., Dvon, L., Walker, E. F. and Chapman, P. (1987). Effect of dietary sunflower oil or hydrogenated coconut oil on growth rate and on swine blood and tissue components of pigs fed fat free diet. *Journal of Nutrition* 92:1903.

Babatunde, G. M., Fajimi, A. O. and Oyejide, A. O. (1992). Rubber seed oil versus palm oil in broiler chicken diets. Effects on performance, nutrients digestibility, haematology and carcass characteristics. *Animal Feed Science and Technology* 35:133-146.

Bah, A. S., Chandhari, S. U. R. and Al-Amin, J. D. (2001). Semen characteristics of local Breeder cocks in the Sahel Region of Nigeria. *Revenue Elev Medical Veterinary Pays Tropical* 54(2): 153-158.

Bains, B. S. (1979). A manual of poultry diseases. Basle, Switzerland: of Holfmann nfLa roche and 10 Ltd. Companies. pp 162-172.

Baker, D. H. and Ammerman, C. B. (1995). Zinc availability: In : Bioavailability of nutrients for animals. (C.B. Ammerman, D.H. Baker & A.J. Lewis, eds). Academic Press, San Diego, pp. 367- 398.

Balinsky, B. I. (1970). An Introduction to Embryology, Philadelphia, W.B. Saunders Co. pp 725.

Balabukh, A.J. and Lyaskovskaya, Y.N. (1980) Lawrie's meat science. Sixth Edition, MyaSna, lndia, S.SR.3:33.

Bao, Y. M., Choct, M., Iji, P. A. and Bruerton, K. (2007). Effect of organically complexed copper, iron, manganese and zinc on broiler performance, mineral

excretion and accumulation in tissues. *Journal of Applied Poultry Research* 16:448-455.

Bao, Y. M. and Choct, M. (2009). Trace mineral nutrition for broiler chickens and prospects of application of organically complexed trace minerals: a review. *Animal Production of Science* 49: 269-282.

Bao, Y. M., Choct, M., Iji, P. A. and Bruerton, K. (2009). Optimal inclusion of organically complexed zinc for broiler chickens. *British Poultry Science* 50: 95-102

Barbato,G. F. (1999). Genetic relationships between selection for growth and reproductive effectiveness. *Poultry Science* 78: 444-452.

Baron, D. N. (1982). Textbook of Chemical Pathology, 4th Ed. Hodder and Stoughton Educational Ltd Kent.

Beattie, J.H. and Kwun, I.S. (2004) Is zinc deficiency a risk factor in atherosclerosis? *British Journal of Nutrition* 9:177–181.

Beattie, J.H., Gordon, M.J., Rucjlidge, G.J., Reid, M.D., Duncan, G.J., Horgan, G.W., Cho, Y.-E. and Kwun,I.S. (2008) Aorta protein networks in marginal and acute zinc deficiency. *Proteonomics* 8: 2126–2135.

Bedford, T. M. and Millar, R. P. (1978). The Character of sperm maturation in the epididymis of the scrotal Hyrex, procavia Capensis and Armadillo, Dasypus move mincintus. *Biology of Reproduction* 19:396-406

Beisel, W. R., Pekarek, R. S., and Wannemacher, R. W. Jr. (1974). Trace element metabolism in animals. In W.G. Hoekstra *et al*., eds.. univ. Park Press, Baltimore, Maryland 2: 217.

Benson, H. J., Gunstream, S. E., Talaro, A. and Talaro, K. P. (1989). Anatomy and Physiology Laboratory Textbook. Win C. Brown Publisher Dubuque IOWA.

Bentic-Smith, A. (1974). Haematology Textbook of Veterinary Clinical Pathology. The Williams and Wilkins Co., Baltimore.

Blom, E. (1983). Sperm Morphology with reference to bull infertility. Ludhiana, First All-India symposium on Animal Reproduction. pp.61-81.

Boland. M. (2003). Trace Minerals in Production and Reproduction in Dairy Cows. *Advances in Dairy Technology* 15: 319.

Boles, J.A., and Shandi, P.J. (2001). Meat cut and injection level affects the tenderness and cook yield of processed Nast beef, *Meat Science*, 59 (3), 259-26.

Bolkent, S., Yanardag, R., Bolkent, S., Mutlu, O., Yildirim, S., Kangawa, K., Minegishi, Y. and Suzuki, H. (2006). The effect of zinc supplementation on ghrelin-immunoreactive cells and lipid parameters in gastrointestinal tissue of

streptozotocin-induced female diabetic rats. *Molecular Cellular Biochemistry* 286: 77-85.

Bradley, B. D., Graber, G., Condon, R. J. and Frobish, L. T. (1983). Effects of graded levels of dietary copper on copper and iron concentrations in swine tissues. *Journal of Animal Science* 56 (3): 625-630.

Bray, P.W, Brieskey, E.S., Cassens, R.G., & Kauffman, R.G. (1969). Techniques and application to meat research. *American Society for Animal Science Research* 221, New York.

Breazile, J. E., Beames, C. G. Jr., Cardielhore, P. T. and Newecomer, W. S. (1971). Textbook of Veterinary Physiology. Lea and Fedbiger. Pub., Philadelphia.

Brillard, J. P. and McDaniel, G. R. (1986). Influence of spermatozoa numbers and insemination frequency on fertility in dwarf broiler breeder hens. *Poultry Science* 65: 2330-2334.

Briz, N. O., Bonet, S., Pinart, B., Egozcue, J. and Camps, R. (1995). Comparative study of Boar sperm coming from the caput, corpus, and cauda regions of the epididymis *Journal of Andrology* 16(2): 175-188.

,

Brocks, J. F. (1961). Recent advances in human nutrition. Little Brown publishers. Boston, Massachusetts. USA.

Brooks, D. E. and Higgins, S. J. (1980). Characterisation and androgen dependence of proteins associated with lumina fluid and spermatozoa in the rat epididymis. *Journal of Reproduction Fertility* 59: 368-375.

Burrell, A. L., Dozier, W. A., Davis, A. J., Compton, M. M., Freeman., M. E., Vendrell., P. F. and Ward, T. L. (2004). Responses of broilers to dietary zinc concentrations and sources in relation to environmental implications. *British Poultry Science* 45: 255-263.

Burrows, W. H. and Quinn, J. P. (1937).The collection of spermatozoa from the domestic fowl and turkey. *Poultry Science* 16:19-24

Burns, R. B. (1983). Antibody production suppressed in the domestic fowl (Gallus domesticus) by zinc deficiency *Avian Pathology* 12: 141-146

Bush, B. M. (1991). Interpretation of Laboratory Results for Small animal clinicians. *Blackwell Scientific publications* 1 (195): 311-317

Cao, J., Henry, P. R. R., Guo, R. A., Holwerda Toth, J. P., Littell, R. C., Miles, R. D. and Ammerman, C. B. (2000). Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. *Journal of Animal Science* 78: 2039-2054

Card, L. E. and Nesheim, M. C. (1972). Poultry production.11th edition, Publisher-Lea nd Febiger Philadelphia. pp. 207-220.

Carlson, M. S., Boren, C. A., Wu, C. E., Huntington, D. W. Bollinger, and Veum, T. L. (2004). Evaluation of various inclusion rates of organic zinc either as polysaccharide or proteinate complex on the growth performance, plasma and excretion of nursery pigs. *Journal of Animal Science* 28:1359–1366.

Carson, J. D., Lorenze, F. W. and Asunndson, V. S. (1995). Semen Production in the turkey male. I. Seasonal Variation. *Poultry Science* 34: 336-343.

Champe, C. P. and Harvey, R. A. (1994). Transamination the funneling of amino groups to glutamate. In Lippincoh illustrated Reviews Biochemistry, 2nd ed. Philadelphia, USA.channel function. In: B.L. O'Dell and R. A. Sunde (eds.) Handbook of characteristics and quality of meat from Bannur Lamb.

Chiboka, O. and Thomas, K. O. (1981). Haematology of West Africa dwarf rams and their crosses with dermer. *Bulletin of Animal Health production in Africa* 29: 325-328.

Church, D. C. and Pond, W. G.(1989). Basic Animal Nutrition and Feeding.3rd ed. John Wiley and Sons, New York.

Church, J. P., Judd, J. T., Young, C. W., Kerbay, J. L. and Kin, W. W. (1984). Relationship among dietary constituents and specific serum clinical component of subject eating self selected diets. *American Journal of clinical Nutrition* 40: 1338-1344.

Chauduri, G. B. (1976). Serum protein pattern in foot and mouth disease in pigs fed with vegetable and animal protein. *Indian Journal of Animal Health* 15 (2): 155:157.

Clulow, J. and Jones, R. C. (1982). Production, transport, maturation, storage and survival of spermatozoa in the male Japanese quail. *Journal of Report Fertility* 64: 259-266.

Coffey, R. D., Cromwell, G. L., Monegue, H. J. (1994). Efficacy of a copper-lysine complex as a growth promotant for weaning pigs. *Journal of Animal Science* 72:2880-2886.

Coles, E. H. (1986). Veterinary Clinical Pathology.4th Ed., W.B. Saunders Company, Philadelphia, USA.

Combs, G. G., Gattis, B. and Shattner, C. S. (1982). Studies with laying hens. 12: Energy restriction. *Poultry Science* 40: 220-224.

Comfort, I. (1994). Colour: It basis and importance. In A. M Pearson and T. R. Duysin eds. Qualityattributes and their measurement in meat. *Poultry and fish*

Products Advances in Meat Research Series. Glasgow: Blackie Academic and Professional. pp 35-77.

Cornelius, C. E. (1970). Liver function in clinical biochemistry of domestic animals. 2nd Edition Kaneko J. l and Cornelius, C. E (Eds). Academic press, New York

Cousins, R. J. and Lee-Ambrose, L. M. (1992). Nuclear zinc uptake and interactions and metallothionein gene expression are influenced by dietary zinc in rats. *Journal of Nutrition* 122: 56–64.

Cousins, R.J., Blanchard, R.K., Moore, J.B., Cui, L., Green, C.L., Liuzzi, J.P., Cao, J. and Bobo, J.A. (2003). Regulation of zinc metabolism and genomic outcomes. *Journal of Nutrition* 133, 1521S–1526S.

Cousins, R.J., Liuzzi, J.P. and Lichten, L.A. (2006) Mammalian zinc transport, trafficking and signals. *Journal of Biological Chemistry* 281: 24085–24089.

Cross, H. R., Savell, J. W. and Francis, J. (1986). National consumer retail beef study. Proceedings of the 38th Annual reciprocal Meat Conference. 39: 112-114.

Dardenne, M. and Bach, J. M (1993). Rationale for mechanism of zinc interation in the immune system. in Nutrient Modulation of the Immune Response. S. Cunningham-Rundles, ed. Marcel Dekker Inc., New York, NY. pp 501–509.

Davice, J. U and Lewis, S. M. (1991). Practical Haematology.8th Ed. pp. 22-68.

David, L. Watts, D. C. (1988). The Nutritional Relationships of Zinc. *Journal of Medicine* 3(2): 63-67.

Davis, G. K. (1980). Microelement interaions of zinc, copper and iron in mammalian species. In: Micronutrient interactions: vitamins, minerals and hazardous elements. eds O.A. Levander and L. Cheng *Annals of the NewYork Academy of Sciences* 355: 130-136.

De kretser, D. M. and Kerr, J. B. (1988). The cytology of the testis. In: The physiology of reproduction (1sted), Knobil, E. and Neill, J. (eds), Raven Press, Ltd; New York. 1:837-932.

De Kretser, D. M., Loveland, K. L. Meinhardt, A. Simorangkir, D. and Wreford, N. (1998). Spermatogenesis. *Human.Reproduction* 13(1): 1-8.

De Reviers, M. and Williams J. (1981). Predicting the adult daily sperm output after the first ejaculation.in cockerels raised under different photoschedules. *Reproduction Nutrition and Development*.21(6B) : 1113-1124.

Delder, A. (1998). Blood and bone marrow. In: veterinary Histology. 5th ed. H.D. *Dellmann and J. Eurell* (Eds) Williams and Wilkins. pp. 62-79.

Dönmez, N. Hüseyin, H., Dönmez, H., Keskin, E. and Çelik, I. (2002). Effects of zinc supplementation to ration on some hematological parameters in broiler chicks. *Journal Biological Trace Element Research Publisher* 87: 1-3 / 125-131.

Dukes, H. H. (1975). Duke's physiology of Domestic Animals 8th Edition.J.thaca and London. canstock publishing Associates. A division of Cornell University Press.

Duncan, J. R., Prasse, K. W. and Mahaffey, E. A. (1994). Veterinary Clinical Pathology 3rd Edition Iowa State University Pres

Dvorak, A. M. and Dvorak, H. F. (1979). The basophil: its morphology, biochemist motility release reactions, recovery and its role in the inflammatory responses of Ige. E- mediated and cell-mediated origin. *Archives of Pathology and Laboratory Medical* 103: 551.

Dzudie, T. (1994). Improvement of quality and consumers acceptability of goat meat by pre and post rigor processing method. Ph.D Thesis University of Ibadan.

Dzudie, T., and Okubanjo, A. (1999). Effects of rigor state and tumbling time on the quality of goat's hams. *Journal of Food Engineer* 4: 103-107.

Eddy, E. M. and O' Brien, D. A. (1994). The spermatozoon. In the Physiology of Reproduction (2nded.) Kobil, E. and Neill, J. (eds), Raven Press, Ltd, New York. 1:29-77.

Egbunike, G. N. and Oluyemi, J. A. (1979). Comparative studies of the reproductive capacity of Nigerian and Exotic Poultry breeds. *Nigerian Journal of Animal Production* 6 (1 and 2): 47-51.

Egbunike, G. N. (1994). *A toast for spermatozoa: One Half the story of mammalian life* (lnaugural Lecture) lbadan. University of lbadan, Nigeria.

Egbunike, G. N., Holtz, W. and Smidt, D. (2007). Reproductive capacity of German Landrance boars: II Sperm production rates as determined by quantitative testicular histology and from gonadal sperm reserves. *Reproduction of Domestic Animals* 11: 35-37.

Eggum, B. O. (1976). Indirect measures of protein adequacy. In: Protein Metabolism and Nutrition. By Cole, T.A., Boorman, K.N. Buttery, P. J., Lewn, D., Nale, R. J. and Wan, H. S. (1976). Butterworth, London-Boston. pp. 249-258.

Eggum, M. E. (1980), Feeds and Nutrition-complete E-mediated and cell-mediated origin. *Archives of Pathology and Laboratory Med*ical 103:551.

Enfalt, A., Kerstin, L. J., Ingerman, H. J., Lundehelm, N. and Enk-mystrom, P. (1997). Effects of outdoors peering and breed (duroc and Yorkshires) on carcass composition, sensory and technological meat quality. *Meat Science* 45 (1): 115.

Epelle, J. J. (1982). Haematolgoical studies in local fowl and their crosses in the parent stock of gold-link chicken. B. Agric Thesis, University of Nigeria

Esiemokhai, O. (1986). Nigerians love cockerel meat (the choice – hard one). Livestock farmers 11(4): 12-13.

Etches, R. J. (1996). Biotechnology and genetic improvement of poultry: Proceedings of the 11th World's Poultry Progress, New Delhi, 1: 295-304.

Etches, R.T. (1998). Reproduction in poultry. CAB Int.,Wallingford, pp: 318

Evans, G. W. and Winter, T. W. (1975). Zinc transport by transferrin in rat portal blood plasma. *Biochemical and Biophysical.Research Communications* 66, 1218-1224.

Ezekwe, A. G., Udozor, I. J. and Osita, C. O. (2003). Effects of quantitative feed restriction on semen quality of Nigerian local cocks. *Nigerian Journal of Animal Production* 30(1):127-132.

Food and Agriculture Organisation of the United Nations (FAO), (2014): Animal Production Health, www.fao.org/ag/againfo/themes.

Fahey, T. D. (1998). Anabolic-androgenic steroids: Mechanism of action and effects on performance. California State University, Chico. USA.

Fetuga, B. L. (1984). Techniques in feed formulation. Paper presented at the feed mill management training workshop held in the Department of Agricultural Economics., University of Ibadan, Nigeria.

Flickinger, C. J. (1981). Regional differences in synthesis, intracellular transport and secretion of protein in mouse epididymis. *Biology of Reproduction* 25:871-883.

Flynn, A., Strain, W. H., Pories, W. H. and Pories, W. J. (1972). Corticotropin Dependency on Zinc ions. *Biochemical and Biophysical.Research Communications* 46 (3): 1113.

Foote, R. H. (1967). Factors prolonging survival of unfrozen bovine spermatozoa. *Journal of Dairy Science* 50 (8): 1338-1340.

Forrest, J. C., Aberle, E. D., Hedrick, H. D., Judge, M. D., and Merkel, R. A. (1975). Principle of Meat Science. San Francisco: W.H Freeman and Co

Frandson, R. O. (1981). Anatomy and physiology of farm animals (7th Ed.) Philadelphia: Lea and Febiger. pp 56-58.

Fraker, P. J., L. E. King, T. Laakko and T. L. Vollmer. (2000). The dynamic link between the integrity of the immune system and zinc status. *Journal of Nutrition* 130:1399S-1406S.

Frimpong, N. A., and Magee, A. C. (1986). Effects of dietary zinc and copper supplementation on serum triglyceride, total-cholesterol and HDL-cholesterol concentrations on young sprague dawley male rats. Sample records for supplementation serum zinc from World Wide Science.org. Accessed 25th January 2014.

Gaynor, M. (1986). Broiler Breeder Dilemma. *Poultry international* 25:12.

Gebriel, G.M., Kalamah, M.A., El-Fiky, A.A. and Ali A.F.A. (2009). Some factors affecting semen quality traits in Norfa cocks *Egyptian Poultry Sciences* 29 (II): 677-693.

Green, J. H. (1972). An Introduction to Human Physiology. Oxford University press, NY.

Guraya, S. S. (1998). Cellular and Molecular Biology of Gonadal Development and Maturation in Mammals. Fundamentals and Biomedical implications: lndia, Narosa publishing House. pp 346.

Hafez, E. S. E. (1993). Physiology of reproduction in poultry In: Reproduction in farm animals (6th Ed.). Philadephia: A & S Febiger. pp 385-402

Hambidge, K. M., Case, C. E., and Krebs, N. F. (1986). In "Trace Element in Human and Animal Nutrition" Academic press, New York.

Harper, A. E. Rodwell, B. A. and Mayes, P. A. (1979). Review of Physiological Chemistry. 17th ed. Lang Medical, Loss Aitos, California pp 60-81.

Harting, T., Steinbach, J. and Smidth, D .C (1981). Investigation of blood in European Pigs raised under tropical conditions. *Animal Research Development* 14:701-706.

Hartoma, T. R., Nahoul, K. and Netter, A. (1977). Zinc, plasma androgens and male sterility. *Lancet* 2:1125-1126.

Hazary, R.C., Wishart, G.J(2001).: Assay of sperm quality in the domestic fowl by MTT reduction. *British Poultry Science* 42: 111–114.

Hays, V. W. and Swenson, M. J. (1985). Minerals and Bones. In: Dukes' Physiology of Domestic Animals, Tenth Edition pp. 449-466.

Health, E. and Olusanya, S. (1985). Anatomy and physiology of tropical livestocks. Longman. 31.

Hedemann, M. S. and Jensen, B. B. (2004). Variations in enzyme activity in stomach and pancreatic tissue and digesta in piglets around weaning. *Archives of Animal Nutrition* 58:47–59.

Hedrick, H. B., Aberle, E.D., Forrest, J. C., Judge, M. D., and Merkel, R. A. (1994). Principle of meat science. (3rd ED.).Dubugne. I.A: Kendal/Hunt publishing company.

Hempe, J. M., Cousins, R. J. (1989). Effect of EDTA and Zinc-methionine complex on zinc absorption by rat intestine. *Journal of Nutrition* 119: 1179-1187.

Henkin, R. L., Schechter, P. J., Hoye, R. and Mattern, C. F. T. (1971). Idiopathic Hypogeusia with Dysgeusia, Hyposmia, and Dysosmia: A new syndrome. *Journal of American Medical Association* 217: 434-440. doi: 10. 1001/jama 1971. 03190040028006

Hess, J. B., Norton, R. A. Downs, K. M. Macklin, K. S. and Bilgili. S. F. (2000). In Trace minerals complexes found to reduce avian cellulitis levels in broiler studies. Trace mineral focus. Zinpro Corp., Eden Prairie, MN. pp 1–6

Hewitt, C. D., Jones, D. J., Savoy, J. and Invills, M. R (1989). Normal biochemical and haematological value in New Zealand white rabbits. *Clinical Chemistry* 35 (8): 1777-1779

Hill, D. A., Peo, E. R., Lewis, A. J., Crenshaw, J. D. (1986). Zinc amino acid complexes for swine. *Journal of Animal Science* 63: 121-130.

Hill, G. M., Cromwell, G. L., Crenshaw, T. D., Dove, C. R., Ewan, R. C., Knabe, D. A., Lewis, A. J., Libal, G. W., Mahan, D. C., Shurson, G. C., Southern, L. L. and Veum, T. L. (2000). Growth promotion effects and plasma changes from feeding high dietaryconcentrations of zinc and copper to weanling pigs (regional study). *Journal of Animal Science* 78: 1010-1016.

Hocking, P. M., Hughes, B. O., and Keer-keers, (1997). Comparison of food intake, rate of consumption, pecking activity and behaviours in layer and broiler breeders male. *British Poultry Science* 38:237-240.

Hoder, M. and Rej, R. (1983). Alanine transaminase. In Methods of Enzymatic Analysis. H.U. Bergmeyer, J. Bergmeyer and M.Grassl (eds.) 3rd Ed.Weinheim, *Verlag Chemie* 3: 416-433).

Hodges, R. D. (1977). Normal avian haematology. In comparative clinical haematology eds. Archer, R.K. and Jeffiot, L. B. Blackwell Scientific Publication, London.

Hoffenberg, R., Black, E. and Block, J. F. (1966).Serum metabolites. *Journal of clinical Investigation* 45:143- 150.

Holtz, W. and Smidt, D. (1976). The fertilizing capacity of epididymis spermatozoa in the pig. *Journal of Reproduction Fertility* 49:227-229.

Huang, Y. L., Lu, L. Luo, X. G. and Liu, B. (2007). An Optimal Dietary Zinc Level of Broiler Chicks Fed a Corn-Soybean Meal Diet. *Poultry Science* 86 (12): 2582-2589.

Huber, A. M., Gershoff, S. N. (1993). Effects of Dietary Zinc and Calcium on the Retention and Distribution of Zinc in Rats Fed Semipurified Diets. *Journal of Nutrition* 72 (2): 296-305.

Hudson, B. P., Dozier III, W.A., Wilson, J. L. Sander, J. E. and Ward, T. L. (2004). Reproductive Performance and Immune Status of Caged Broiler Breeder Hens Provided Diets Supplemented with Either Inorganic or Organic Sources of Zinc from Hatching to 65 wk of age. *Journal of Applied Poultry Reserve* 13: 349-359.

Hullberg, A., Johannsson, L. and Lunstram, K. (2005). Effects of tumbling and RN genotype on sensory perception of cured-smoked pork.Meat science. In: press, doi: *Meat Science* 2005, 69 (4): 721-732 doi: 10. 1016/j.meatsci 2004.07.013.

Hunton, P. (1986). Importance of water in pullet and egg production. *Poultry International Journal* Watt. Publication. pp 42-48.

Ibe, S. N. and Obi, J. C. (1986). Effect of dosage of undiluted semen and frequency of insemination on fertility of chicken eggs. *Nigerian Journal of Animal Production. Proceedings* 11th Annual Conference of Nigeria Society of Animal Production., A.B.U., Zaira. March 23rd-27th.

Ibs, K. H. and Rink. L. (2003). Zinc-altered immune function. *Journal of Nutrition* 133: 1452S-1456S.

Idowu, O. M. O., Ajuwon, R. O., Oso, A. O. and Akinloye, O. A.(2011). Effects of Zinc Supplementation on Laying Performance, Serum Chemistry and Zn Residue in Tibia Bone, Liver, Excreta and Egg Shell of Laying Hens. Asian Network for Scientific Information. *International Journal of Poultry Science* 10 (3): 225-230.

Ihekeronye, A. and Ngoddy, P. O. (1986). Integrated food science and technology for tropics. London and Basingstroke: Macmillan.

Ikeme, A. (1990). Meat science and technology.A comprehensive approach. Onitsha: Africa-Fep. Publishers Limited.

International Zinc Association (2011) Zinc essential for life, History of Zinc, Greg Brouwer, Director: Zinc Nutritive Initiative. www.zinc.org. Assessed on 2nd February 2014.

Iyayi, E. and Tewe, O. (1998). Serum total protein, urea and creatine levels as indices of quality of cassava diets for pigs. *Tropical Veterinary* 16: 59-67.

Jain, N. C. (1986). Schalms Veterinary Haematology. 4th Ed. Eea and Febiger, Philadelphia, USA.

Jamudeen, M. R., Bongso., T. A. and Dass, S. (1982). Semen Characteristics of the swamp Buffalo (Bubalus Bubali). *Animal Reproduction Science* 4:213

Jenner, A., Ren, B., Rajendran, R., Ning, P., Huat, B. T. K, Watt, F., Halliwell, B. (2007) Zinc supplementation inhibits lipid peroxidation and the development of

atherosclerosis in rabbits fed a high cholesterol diet. *Free Radical Biology and Medicine* 42:559 –566.

Johnson, P. E. (1982). A mass spectrometric method for use of stable isotopes as tracers in studies of iron, zinc and copper absorption in human subjects. *Journal of Nutrition* 112:1414-1424.

Johnson, A. B., and T. M. Fakler. (1998). Trace minerals in swine and poultry nutrition. In Technical Bulletin. Zinpro Corp., Eden Prairie, MN. pp 1–24

Jondreville, C. and Revy, P. S. (2003). An update on use of organic minerals in swine nutrition. In Proceedings of Eastern Nutrition Conference. Quebec, Canada. pp 1-16.

Jones, D. G. (1993). The eosinophils: Review article *Journal of Comparative Pathology* 108: 317-335.

Kaplan, A. and Szabo, L. L. (1983). Clinical Chemistry. Interpretation and Techniques. 2nd Edition. Lea and Febiger. Philadelphia.

Katz, D. F. (1983). The Evolution of Mammalian Sperm Motility in the Male and Female Reproductive Tracts. In: The sperm Cell, Andre J. (ed), Martinus Nijhoff Publishers, Boston. pp 340-344.

Keilin, D. and Mann, T. (1940) Carbonic anhydrase. Purification and nature of the enzyme. *Biological Journal* 34, 1163–1176.

Kerr, G. R., Lee, E. S., Man M. K., Lovinas, R. J., Randoll, E., and Foot holder. R. N. (1982). Relationship between dietary and biochemical Measure of Nutrition Status. *American Journal of Clinical Nutrition* 38: 294-318.

Kerr, M. G. (1979). Veterinary Laboratory.Medicine clinical biochemistry and haematology. pp 35-63.

Keshavarz, (1984). Reduce dietary protein without decreasing egg production. *Poultry International Journal*, 70.

Kessler, J. I. Morel, P. A. Dufey, A. Gutzwiller, A. S. and Geyer, H. (2003). Effect of organic zinc sources on performance, zinc status and carcass, meat and quality in fattening bulls. *Livestock Production* Science 81:161-171.

Kidd, M. T., Qureshi, M. A. Ferket, P. R. and Thomas. L. N (1994). Blood clearance of *Escherichia coli* and evaluation of mononuclear-phagocytic system as influenced by supplemental dietary zinc-methionine in young turkeys. *Poultry Science* 73:1381–1389.

Kidd, M. T., Ferket, P. R. and Qureshi. M. A. (1996). Zinc metabolism with special reference to its role in immunity. *World's Poultry Science Journal* 52:309-323

King, J. C. and Turnlund, J. R. (1989). Human zinc requirements. In: Zinc in human biology, ed. C.F. Mills, 335-350. New York: Springer-Verlag. (cited by Cousins, 1996)

King, L. M., Holsberger, D. R., Donoghue, A. M. (2000). Correlation of CASA velocity and linearity parameters with sperm mobility phenotype in turkeys. *Journal of Andrology* 21; 2000 (1) 65-71.

King, L.M., Kirby, J.D., Froman, D.P., Sonstegard, T.S., Harry, D.E., Darden, J.R., Marini, P.J., Walker, R.M., Rhoads, M.L., Donoghue, A.M.(2000) Efficacy of sperm mobility assessment in commercial flocks and the relationship of sperm mobility and insemination dose with fertility in turkeys. *Poultry Science* 79:1797–1802.

Kirchgessner, M. and Roth, J. P. (1981). In ''Proc Trace Element Metabolism in Man and Animals (TEMA-4)'' Canberra, Australia. *Australian Academy of Science* pp 32-85

Kohn, R.A.and Allen, M.S. (1995). Enrichment of proteolytic activity relative to nitrogen in preparations from the rumen for in vitro studies. *Animal Feed Science Technology* 52(1/2): 1-14.

Kreager, K. (1988). Management of feed energy for pullets and layers.Technical Bulletin.A Publication of Hy-Line International.

Kronfeld, D. J. and Medway, W. (1975). Blood Chemistry In: Textbook of Veterinary Clinical Pathology. Williams M., James, E. P. and John, S.W. The Williams and Wilkins Co.,Baltimore.

Krop, D. H (1980). Effect of retail display conditions on meat colour. In: Proceedings of 33rd Recipe Meat Conference. West Lafayelter. pp 15-32.

Kruger, T., Menkveld, R., Stander, F., Lombard, C. J., Vander Merwe, J., Van Zyl, J. and Smith, K. (1986). Sperm Morphologic Features as a prognostic factor in *In vitro* Fertization. *Fertility and Sterility* 46: 1118-1123.

Kubena, L. F., May, J. D., Peace, F. N. and Deaton, J. W. (1972). Haematocrit and haemoglobin of broilers as influenced by environmental temperature and dietary iron level. *Poultry Science* 51:759.

Kucuk, O., Sahin, N. and Sahin, K. (2003): Supplemental zinc and vitamin A can alleviate negative effects of heat stress in broiler chickens. *Biological Trace Element Research* 94: 225-235.

Kuster, C.E., Singer, R.S., Althouse, G.C. (2004): Determining sample size for the morphological assessment of sperm. *Theriogenology* 61: 691–703.

Kwun, I. S., Cho, Y.E., Lomeda, R.A.R., Kwon, S.T., Kim, Y. and Beattie, J.H. (2007) Marginal zinc deficiency in rats decreases leptin expression independently of food intake and corticotrophin-releasing hormone in relation to food intake. *British Journal of Nutrition* 98, 485–489.

Laing, J. A. .(1979). Fertility and Infertility in domestic animals.3rd Edn. London, Bailliére Tindall,

Laird, C. W., Fox, R .R.., Mitchell, B. P., Blau, E. M. and Schultz, H. S. (1970). Effects of strain and age on some haematological parameters in the rabbits. *American Journal of Physiology* 218:1613-1617.

Lake, P.E. (1971). The male in reproduction. In:Physiology and Biochemistry of the Domestic Fowl. D.J. Bell and B.M. Freeman (eds.), 3: 1411-1447.

Lake, P. E. and Stewart, J. M. (1978). Artificial insemination in poultry. Bulletin, Ministry of Agriculture, Fisheries and Food, No. 213.

Larry, L. B. (1987). Salt and Trace Minerals for Livestock, Poultry and Other Animals. Published by the Salt Institute 700 North Fairfax Street, Suite 600 Alexandria, Virginia. pp 2231-2240.

Latner, A. L (1975). In: Catherow and Trumper. Clinical biochemistry.7th E d.W. B Sunders, Philadelphia.

LeeDec, M. C., Suka, J. and Skrobanek, P. (1981). *Animal Breeding Abstract* 49 (7): 4284.

Lesson, S. (1990). Energy intake for leghorn.Technical Bulletin. A publication of Hy-Line inters Livestock Farmers. *Journal of Food Science and Technology* 11 (4):12 – 13.

Leeson, S. (2003): A new look at trace mineral nutriton of poultry: Can we reduce the environmental burden of poultry manure? In, Lyson T. P., Jaques, K. A (Eds): Nutritional Biotechnology in the Feed and Food Industries. Nottingham University Press, Nottingham UK. pp. 125-129.

Leeson, S., (2005). Trace mineral requirements of poultry–validity of the NRC recommendations. Published in "Re-defining Mineral Nutrition" edited by J. A Taylor-Pickard and L. A. Tucker, Nottingham University Press, Nottingham, United Kingdom. pp 107-117.

Leeson, S. and Summers. J. D. (2001). Scott's Nutrition of the Chicken. 4th Ed. University Books, Guelph, Ontario.

Legg, S. P. and Sears, l. (1980). Trace element Metabolism. *Animal Proceeding International. Symposium* ,2nd, pp 628

Levengood, J. M., Sanderson, G. C., Anderson, W. L., Foley, G. L., Brown, P. W. and Seets, J. W (2000). Influence Of Diet On The Hematology And Serum

Biochemistry Of Zinc- Intoxicated Mallards. *Journal of Wildlife Diseases* 36(1): 111-123.

Linares, L.B., Broomhead, J.N., Guaiume, E.A., Ledoux, D.R., Veum, T.L. and Raboy, V. (2007) Effects of a low phytate barley (*Hordeum vulgare* L.) on zinc utilisation in young broiler chicks. *Poultry Science* 86: 299–308.

Longe, O. G. (1984). Fibre in Layers diet. *British Poultry Science* 25:187.

Lowel, J. A., Wiseman, J. and Cole, D. J. A. (1994). Absorption and retention of zinc when administered as an amino -acid chelate in the dog. *Journal of Nutrition* 124: 2572-2574

Luscombe, N. M., Austin, S. E., Berman, H. M. and Thornton, J. M. (2000). Review: An overview of the structures of protein-DNA complexes. *Genome Biology* 1: 1-10.

Magumdar, N.N (1980). A textbook of histology Vikas publishing House PUT LTD India.on fertility in dairy cattle. *Animal Reproduction of Science* 96: 240-249.

Mahendrakar, N. S., Khabade, V. S. and Dani, N. P. (1988).Studies on the effect of Fattening or Carcass characteristics and quality of meat from Bannur Lamb. *Journal of Food Science Technology* 25: 228-231.

Malik, A., Haron, A. B. D., Yusoff, R., Nesa, M., Bukar, M. and Kasim, A. (2013). Evaluation of the ejaculate quality of the red jungle fowl, domestic chicken, and bantam chicken in Malaysia. *Turkish Journal of Veterinary and Animal Science* 37: 564-568 doi:10.3906/vet-1107-26

Mann, T. (1974). Effects of Nutrition on male accessory organs. In: male accessory sex organs. D. Brnades (ed) New York, Acad. Press.

Mass, J. A., Metrose, D. R., Reed, H. C. D. and Vandeplassche, (1979). Semen characteristics and infertility in domestic animals (Laing. J.A. ed) 3rd ed. pp. 59-91.

Mattew, N.I. & Robert, M.E. (1990). Principles of Physiology. Wolf Publishers Ltd. 181-191.

Maxwell, H. M., Robertson, W., Spence, S. and Marchorquo-dale, C. C. (1990). Comparison of haematological parameters in restricted and *adlibitum* fed domestic fowls. *British Poultry Science* 31:407-413.

McClain, C. J., Gavaler, J. S. and Van Thiel, D. H. (1984). Hipogonadism in the Zinc deficient rat: localization of the functional abnormalities. *Journal of Laboratory Clinical Medicine* 104:1007-10015

McDonald, P., Edwads, R. A., Green halgh J. F. O., and Morgan, A. (1995). Animal Nutrition 5th Edition Longman Group Ltd, London.

McDowell, L. R. (2003). Zinc Minerals in Animal and Human Nutrition. Elsevier Science, Amsterdam, the Netherlands. pp 357–396.

McCartney, M. (1999). Reproductive System of a Rooster Wikipedia on Ask.com http://www.chacha.com/question/where-is-a-rooster. Accessed on 25th November, 2013.

McCartney, E. (2008). Trace minerals in poultry nutrition--sourcing safe minerals organically? *World Poultry* 24: 2, 14-15.

McLaren, D. S (1988). A fresh look at protein- energy malnutrition in hosipitalized patient. *Nutrition* 4: 1-6

Meftah, S. P. (1984). Testicular androgen binding protein in zinc deficient rats. *Nutrition Rex* 4: 4374

Merck VM (1986). The Merck Veterinary Manual. Sixth Edition. A handbook of diagnosis, therapy and disease prevention and control for the veterinarian. Published by Merck and Co., Inc., Rahway, NewJersey, USA.

Miles, R. D. and Henry, P. R. (2000).Relative trace mineral bioavailability. Ciencia *Animal Brasileira* 1(2):73

Mills, C.F., Dalgarno, A.C., Williams, R.B. and Quarterman, J. (1967) Zinc deficiency and the zinc requirements of calves and lambs. *British Journal of Nutrition* 21, 751–768.

Miller .W. J. (1970). Zinc Nutrition of Cattle: A review. *Journal of Dairy Science* 53 (8): 1123-1135

Mitruka, B. M. and Rawnsley, H. M. (1981). Clinical Biochemical and Haematological Reference Values in Normal Experimental Animals. *Masson Publisher U.S.A. Inc*., N.Y. pp 21-84.

Mohanna, C. and Nys., Y.*(* 1999). Effect of dietary zinc content and sources on the growth, body zinc deposition and retention, zinc excretion and immune response in chickens. *British Poultry Science 40*:*108*–114.

Moore, H. D. M. (1995). Post-testicular sperm maturation and transport in the excurrent ducts. In Gametes.The spermatozoon, Grudinskas, J.G. and Yorich, J.L. (eds.), Cambridge University press, Cambridge, Great Britain. pp. 140-156.

Morag, G. (1989). Interpretation of Scientific Veterinary Laboratory Medicine Tpst and clinical biochemistry and haematology. *Black Well Science Pub* 1st Ed. pp. 67-109.

Moss, J. A., Metrose, D. R., Reed, H. C. D. and Vandeplassche, (1979). Semen characteristics in fertility and infertility in domestic animals (Laing. J.A.ed) 3rd ed. pp 59-91.

Murray, R. K., Granner, O. K., Mayers, P. A. and Rodwell, V. M. (1988). Harpers Biochemistry. 21st ed. Appleton and Large Norwalk (Pub) Connective/ Sanmateo, California. pp: 649-664.

Murray R. K., Granner, D. K., Mayes, P. A, Rodwell, V. W (2000). Harper's Biochemistry, 25th Edition, McGraw-Hill, Health Profession Division, USA.

Nagabhushanam, R., Kodarkar, M. S. Sarojini, R. (2002). Textbook of Animal Physiology, 2nd Ed., Oxford and IBH Publishing Co.Pvt. Ltd., New Delhi. pp 634.

National Research Council (NRC) (1980). Mineral Tolerance of domestic Animals. Subcommittee on mineral Toxicity in Animals. National Academy of Science.Washington, D.C.

National Research Council (NRC) (1994). Nutrient Requirement of Poultry. 9th revised. National Academy Press.Washington, D.C.

National Research Council (NRC) (1998).Nutrient requirement of swine.10th ed. National Acad. Press Washington, DC.

Naz, R. K and Minhas, B. S. (1995). Enhancement of sperm functions for treatment of male infertility. *Journal of Andrology* 16 (5): 384-388.

Niola, D., and Salis M. (2011) Zinc metabolism Pathway detail - flipper e nuvola 2011-12-29 T11:43:57. Gianpiero Pescarmona.

Nissi, J. (2004) .Serum Protein electrophoresis: Primary Medicinal Reviewers. Patrice Burgess.

Njike, M. C (1979). Crude Protein requirement of growing chickens in Nigeria Environment. *Nigerian Journal of Animal Production* (1and 2): 52-58.

Nkanga, E. E. (1989). Breed and Seasonal influences on the reproductive potential of the cocks in a humid tropical environment. Ph. D. Thesis, Animal Science, Dept., University of Ibadan.

Nkanga, E. E. and Egbunike, G. N. (1990).Cytology and the cycle of the seminiferous epithelium in the cock (Gallus domesticus). *Tropical Animal Production Invest* 1:88-100.

Nollet, L., Van der Klis J. D., Lensing M., and Spring, P. (2007). The Effect of Replacing Inorganic With Organic Trace Minerals in Broiler Diets on Productive Performance and Mineral Excretion. *Journal of Applied Poultry Research* 16: 592-597.

Nollet, L., Huyghebaert, G. and Spring, P. (2008). Effect of Different Levels of Dietary Organic (Bioplex) Trace Minerals on Live Performance of Broiler Chickens by Growth Phases. *Poultry Science Association* 17: 109–115

Nwagu, B. I., Abubakar, B.Y., Oni, O. O., Adeyinka, I. A. and Egbuedo, U. C. (1996). Semen Characteristics of Rhode Island Red *and* White Breeder Cocks. *Nigerian Journal of Animal Production* 23:15

Nwakalor, L. N., Okere, G. C. and Njoku, D. C. (1986). Semen characteristics of guinea fowl(Numida meleagris) *Journal of Animal Production Proceedings* Ahmadu Bello University Zaria March, 23-27.

Obidi, J. A., Onyeanusi, B. I., Ayo, J. O. Rekwotand, P. I. and Dzenda, T. (2008). Determination of Gonadal Sperm/Spermatid Reserves in Shikabrown Breeder Cocks. *International Journal of Poultry Science* 7 (12): 1200-1203.

O'Dell, B.L. and Savage. J.E. (1957) Symptoms of zinc deficiency in the chick. *Federation Proceedings* 16: 394.

O' Dell, B. L. (1981). In "Proc. Trace Element Metabolism in man and Animals (TEMA – 4)" (J.M.C. Howell, J.M. Gawthrone, and C.L White eds), *Australiam Academy of Science* Canberro, Australian. pp. 319.

O'Dell, B. L. (1997). Mineral-ion interaction as assessed by bioavailability and ion channel function. In: B.L. O'Dell and R. A. Sunde (eds.) Handbook of nutritionally, essential mineral elements. Marcel Dekker, Inc., New York. pp.64

Obioha, F. C., Asuquo, B. O. and Igbo, D. (1982). Optimum protein and energy levels of local and exotic egg type chickens. *Nigerian Journal of Animal Production* 9: 35-43.

Oguike, M. A., Ndubueze, A. N and Ibe, S. N. (2000). Semen quality of different genotypes of Nigerian local cocks. *Journal of Sustainable Agriculture and Environment* 2(2):310-313.

Olayemi O., Oyewale, J. O. and Omolewa, O. F. (2002). Plasma chemistry values in the young and adult Nigerian duck (Anas Platyrhynchos). *Israel Journal of Veterinary Medicine* 57(4):156-158.

Olomu, J. M. (1979). Poultry Nutrition Research: It's contribution to National Poultry Industry. Nutrient requirements, nutrient source and nutrient content of feed ingredients. *Proceedings of National Seminar of Poultry Production*. In Nigeria. Ahmadu Bello University, Zaria.

Olomu, J. M. (1995). Monogastric animal nutrition. Principles and practice (1st Ed). Jachem Publication.

Olorede, B. R., Ajagbonna, O. P. and Babatunde, G. M. (1995). Comparison of air dried poultry droppings in broiler rations. Effects on performances, organ weights and haematological parameters. *International Journal of Animal Science* 10: 289-293.

Olson, G. E. and Danzo, B. J. (1981). Surface changes in rat spermatozoa during epididydmal transit. *Biology of Reproduction*. 24: 431-443.

Oluyemi, J. A. and Roberts, F. A. (1979). Poultry Production in Warm Wet Climate.(1st Ed). The Macmillan Press Ltd., London.

Oluyemi, J. A. and Roberts, F. A. (2000). Poultry production in warm wet climates.Published by spectrum books Ltd. Ibadan, Nigeria, 2nd ed. pp.1-7.

Onifade, A. A. and Tewe, O. O. (1993). Alternative tropical energy feed resources in rabbit diet growth performance, diet digestibility and blood composition. *World Rabbit Science* 1: 17-24.

Orji, B. L., Okeke, G. C. and Akunyiba, A. O. (1987). Haematological studies on the Guinea fowl (Numida meleagris pallas). Effects of age, sex and time of bleeding on the haematological values of guinea fowls. *Nigeria Journal of Animal Production* 13: 96-101.

Osborne, C. A., Low, D. G. and Finco, D. R. (1972).Canine and Feline Urology. W.B. Saunders, Philadelphia. In: Veterinary Clinical Pathology by Coles. pp. 262-263.

Osineye, O. M. (2009). Effect of Dietary Zinc supplements on growth and pregnancy in West African Dwarf Goat. Ph.d Thesis. University of Ibadan, Nigeria

Osinowo O., Osori, D. I. K. and Molokwu, E. C. I (1981).Growth and testicular development in Bunaji Bulls. *Journal of Animal Production Research* 1: 55

Ott, E.A., Smith, W.H., Stob, M. and Beeson, W.M. (1964) Zinc deficiency syndrome in young lamb. *Journal of Nutrition* 82, 41–50.

Ott, E. A., Smith, W. H., Harrington, R. B., Stob, M., Parker, H. E., and Beeson, W.M. (1966). Zinc toxicity in Ruminants IV. Physiological changes in the tissue of beef cattle. *Journal of Animal Science* 25:432-438

Oyewale, J. O. and Ogwuebu, S. O. (1986). Haematological studies on the guinea fowl (Numida meleagris pallas). *Bulletin of Animal Health Production of Africa* 34: 61-65.

Oyewale, J. O. (1987). Haematological studies in apparently healthy Nigerian domestic chicken (Gallus domesticus). *Bullentin of Animal Health Prod*uction of *Africa* 35:108-112

Oyewale, J. O., Olowookorun, M. O., Abatan, M. O. and Fajimi, J. L. (1988). Serum electrolyte and protein levels in guinea- fowls (Numida meleagris) and Nigeria fowls (Gallus domesticus). Beitrage zur tropischen Landwirtschaft und Veterinarmedizin, 26:81-85.

Oyeyemi, M. O., Akusu, M. O., Olaoye, M. O. and Omobowale, O. T. (1996). Effect of frequent ejaculation on the semen characteristics of West African Dwarf bucks. *Tropical Veterinary* 14:71-75.

Parák, T., and Strakova, E. (2011). Zinc as a feed supplement and its impact on plasma cholesterol concentrations in breeding cocks. *Acta Veterinary* 80: 281–285;

Patrick, H. and Schaible, A. J. (1980). Poultry feed nutrition (2nd ed.) Publishing company, Inc.

Pearson, A. M. (1994). Quality attributes and their measurement in meat, poultry and fish products. In G.A.M Pearson and T.R Dutson (eds.) Advances in meat research studies. Glasgow: Blackie Academi Professional Press.

Peters, T., Biamonte, G. T. and Doumas, B. T. (1982). Protein (Total Protein) in serum urine and cerebrospinal fluid, albumin in serum.In selected method of clinical chemistry. Vol 9, (W.R. Paulkner and S. Meites, Eds) *American Association for Clinical Chemistry* Washington, D.C.

Peters, S. O., Shoyebo, B. M., Ilori, O. D., Ozoje, M., Ikeobi, C. O. N and Adebambo, O. A (2008). Semen Quality traits of seven strains of chicken raised in the Humid Tropics. *International Journal of Poultry Science* 7(10): 949-953

Peric, L., Nollet, L., Miloševic, N. and Zikic, D. (2007). Effect of Bioplex and Sel-Plex substituting inorganic trace mineral sources on performance of broilers. *Archi fur Geflügelkunde* 71 (3):,122–129.

Pfaff, W., Pescature, A. J., Putz, F., Cantor, A. H. and Johnson, T. H. (1990). Effect of frequency of semen collection and semen production and fertility during post-rest period in poultry. *Poultry Science Abstract* 69:183

Philipel, T. (1970). Relation between reproductive capacity and semen quality in cocks. Ptisevidstvo 20(6): 32-34.

Pimental, J. L., Cook, M. E and. Greger. J. L (1991). Immune response of chicks fed various levels of zinc. *Poultry Science* 70: 947–954.

Polakoski, K. L. and Zaneveid, L. J. D. (1977). Biochemical examination of the human ejaculation.In Techniques of Human Andrology. E.S.E Hafez (ed). New York, North-Holland Publication Co.

Pories, W. J., Henzel, J. H. and Hennessen, J. A. (1968). Trace Substance in Environmental Health-, Columbia, *Proceedings of University Mo Annual Conf*erence 1: 114.

Poultry study guide (2004): Glossary terms, Small Animal Expo-Madera Country 4-H, level 1 and level 11, Avian American system of judging.

Praps, E. L. and Birds, H. R. (1983). Nutrient requirement of Poultry. *Feed Stuff* 26:2-30.

Prasad, A. S., Schoemaker, E. B., Ortega, J., Brewer, G. J., Oberleas, D. and Oelshlegel, F. J. (1975). Zinc Deficiency in Sickle Cell Disease. *Clinical Chemistry* 21: 2

Prasad, A.S. (1993). Biochemistry of Zinc. Plenum Press, New York

Prasad, A.S. (2009) Zinc: role in immunity, oxidative stress and chronic inflammation *Clinical Nutrition and Metabolic Care*, 12:646–652.

Price, J. F and Schweigert, B. S. (1972). The Science of meat and meat products (2nd ed.) Sanfrancisco: W.H Freeman and Company.75: 610.

Quarterman, J. and Florence E., (1972). Observations on Glucose Tolerance and plasma levels of free fatty acids and insulin in the Zinc- deficient rat. *British Journal of Nutrition* 28 (01): 75 -79.

Ranjkan, B. K. (1981). Animal Nutrition in the tropics, 2nd ed. Vikas Publishing house, P.U.T., Ltd.

Rahman. Jn., Hasan, N. M. Abbas, R. (2008). Improved broiler chick performance by dietary supplementation of organic zinc sources. *Asian - Australasian Journal of Animal Sciences* 21(9): 1348-135.

Rath, N. C., Balog, J. M., Huff, W. E., Huff, G. R., Kulkarni G. B., and Tierce. J. F. (1999). Comparative differences in the composition and biomechanical properties of tibiae of seven-and seventy-two-week-old male and female broiler breeder chickens. *Poultry Science* 78: 1232-1239.

Reid, B. C. and Weber, C. W. (1975). Supplemental Dietary fat and laying hen performance. *Poultry science* 54: 422-428.

Reid, W. M., Pesti, G. M. and Hammerhund, M. (1990). Raising healthy Poultry under primitive condition. *A publication of Christian Veterinary Mission* pp 49-57.

Reiterer, G., Macdonald, R., Browning, J., Morrow, J., Matveev, S.V., Daugherty, A., Smart, E., Toborek, M. and Hennig, B. (2005). Zinc deficiency increases plasma lipids and atherosclerotic markers in LDL-receptor-deficient mice. *Journal of Nutrition* 135: 2114-2118.

Rekwot, P. I., Lamidi, O. S., Adamu, A. M., Egbuedo, C. U., Ruwaan, J. S., and Okereke, S. N. (1987). Reproductive performanceof Bunaji bulls grazing natural pasture and receiving supplements containing palm kernel meal. *Nigerian Veterinary Journal* 18: 26-36.

Ren, M., Rajendran, R., Ning, P., Tan Kwong Huat, B., Choon Nam, O., Watt, F., Jenner, A. and Halliwell, B. (2006). Zinc supplementation ccccccccccccd decreases the development of atherosclerosis in rabbits. *Free Radicals Biology of Medicine* 41: 222-225.

Rhodimet, T. M. (1993). Feed formulation guides (6th Ed). London: Macmillan.

Ritzen E. M. (1983). Chemical messengers between Sertoli cells and neighbouring cells. *Journal of Steroid Biochemistry* 19(1): 499-504.

Robaire, B. and Hermo, L. (1988). Efferent Ducts. Epididymis, and vas deferens structure, functions, and their regulation. In: the physiology of Reproduction. Knobil, E. and Neill, J. (eds.), Raven press, Ltd; New York, 1:999-1080.

Robinson, M. F., Mckenzie, J. M., Thompson, C. D. and Van Rij, A. L., (1973) Metabolic balance of zinc, copper, cadmium, iron, Molybdenum and selenium in young New Zealand women. *British Journal of Nutrution* 30:195-205

Romero-Sanchez, H., Plumstead, P. W and Brake, J. (2007). Feeding broiler breeder males. 1. Effect of feeding program and dietary crude protein during rearing on body weight and fertility of broiler breeder males. *Poultry Science* 86: 168-174.

Ross, J. G., Christie, G., Halliday, W. G. and Jones, R. M. (1978).Haematological and blood chemistry comparison values for clinical pathology in *Poultry Veterinary Record* 102: 29-31.

Rossi, P., Rutz, F., Anciuti, M. A., Rech, J. L and Zuk, N. H. F (2007).Influence of Graded Levels of Organic Zinc on Growth Performance and Carcass Traits of Broilers. *Journal of Applied Poultry Research* 16: 219-223.

Rowett, H. G. O. (1962). Histology and Embryology. 2nd ed. John Murry (Pub) Ltd., London. pp 72.

Russell, L. L., Ettlin, R. A., Sinha Itikim., A. P. and Clerg, E. D. (1990). Histological and Histopathological evaluation of the testis. Cache River press. pp 286.

Ryan, J. P., Kearns, P. and Quinn, T. (2002) Bioavailability of dietary copper and zinc in adult Texel sheep A comparative study of the effects of sulfate and Bioplex supplementation. *Irish Veterinary Journal* 55 (5): 221- 224.

Saez, J. N. (1994). Leydig cell.Endocrine, Paracrine and Autocrine Regulation. *Endocrinology. Revenue* 15:574-626.

Saacke, R. G. (1982). Components of Semen Quality. *Journal of Animal Science*. 55 (ll):1-13.

Saied, J. M. and Al Soudi, K. A. (1975). Seasonal Variation in semen characteristics of white leghorn, New Hampshire and Indigenous chicken in Iraq. *British Poultry Science* 16:103-113.

Salako, A. O. (2001). Response of Gilts to Neem leaf-based diets. Effects on serum biochemistry, kidneys and lungs. B.Sc. Project report. Department of Animal Science, University of Ibadan, Nigeria.

Sandstead, H. H., and Evans, G. W. (1984) In "Nutrition Reviews present knowledge in Nutrition" 5th ed. (R.E. Olson, Ed). The Nutrtiion foundation, Inc., Washington, D.C. p. 479

Sandstrom, B. (1992). Dose dependence of zinc and magnesium absorption in man. *Proceedings of the Nutrition Society* 51:211-218.

Sarror, O. W. and Vanveen Schillhorn, T. N. (1977). Hematological values of Udah Yankasa sheep in the Northern guinea savannah of Nigeria. *Tropical Animal Health Production*. 245-248.

Sauer, Bryan G., (1998). "Effects of Zinc on Glucose-6-Phosphate Dehydrogenase in Buffalo Rat Liver Cells" *Honors Theses*. Paper 250. http://scholarworks.wmich.edu/honors_theses/250

Say, R. R. (1987). Manual of Poultry Production in the Tropics. (AB International). Wallingford. U.K 52-67.

Scan, (2003). Opinion of the Scientific Committee for Animal Nutrition on the use of zinc in feedingstuffs. http://ec.europa.eu/food/fs/sc/scan/index_en.html Commission Regulation EC No 1334 2003 of 25 July 2003.

Schalm, O. W. (1971). Clinical significance of plasma protein concentration. *Journal of American Veterinary Medical Association* 157:1672.

Schalm, O. W., Jain, N. C. and Carol, E. J. (1975) Veterinary Haematology, 3rd Ed., Lea and Febiger, Philadelphia. pp 340-370.

Schemer, S. (1967). The blood morphology of laboratory animals. In: Clinical Biochemical and Haematological Reference Values in Normal Experimental Animals. B.M. Mitruka and H.M. Rawnsley (eds) Masson Pub. USA Inc., N.Y. pp.84.

Scott, M. L. (1974). Poultry feeding and management in the tropics. Animal production in the tropics edited by J. L. Loosli, V. A. Oyenuga and G. M. Babatunde. Published by Heinemann Educational Books. pp. 200-212.

Scott, M. L., Nesheim, M. C. and Young, R. J. (1982). Nutrition of the Chicken, 3rd Edition.M. L. Scott and Associates, Ithaca, NY.

Semen Analysis (2007). From Wikipedia, the free encyclopedia) "Understanding Semen Analysis". Stonybrook, State University of New York. Retrieved 2007-08-05.

Sidhu, P., Gorg, M. L., Morgenstern, P., Vogt, J., Butz, T., Dhawan, D. K (2004). Role of Zinc in regulating the levels of hepatic elements following nickel toxicity in rats. Biological Trace Elements Research. 102: 161-172.

Sigma, (1991) Diagnostic kits and reagent. Sigma chemical com. St. lois Mo. USA.

Skinner, J. T., Waldroup, A. L., and WaldrouP, P. W. (1992). Effects of removal of vitamins and trace mineral supplements from grower and finisher diets on live performance and carcass composition of broilers. *Journal of Applied Poultry Research* 1: 280-286.

Small and Backyard Flocks, (2012). External Anatomy of Poultry Kept on Small or Backyard Flocks: Chicken http://www.extension.org/poultry. Assessed 2nd November 2014.

Smith, A. J. (1991). Poultry, the agriculturist. CTA series *Macmillann Press Ltd.* London. pp. 162- 191.

Smulders, F. M., Vanlaack, Eikelenboom R. E. G. (1991) . In F. Smolders (ed.). The Europe meat industry in 1900s. Andef, Nigmegen. pp 359.

Soetan, K. O., Olaiya C. O. and. Oyewole O. E. (2010). The importance of mineral elements for humans, domestic animals and plants: A review. African *Journal of Food Science* 4: (5) pp 200-222

Statistical Analysis Systems SAS –Institute Incs. (2001). SAS Campus Drive Cary, North Caroline U.S.A.

Stanton, T. L., Pas, D., Schutz, and Swenson, C. (2008).T race Mineral Supplementationin the Presence of Antagonists on Growth Performance, Health, and CarcassCharacteristics of Transport-Stressed Calves. Department of Animal Sciences, Colorado State University, Fort Collins, CO 80523-1171. *The Professional Animal Scientist* 17:101–108

Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo J. C, Witztum, J. L. (1989). Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *New England Journal of Medicine* 320: 915–92

Stevenson, Z. (2009). The Essence of mineral nutrition for ducks .Alltech European Bioscience Research Centre, Dunboyne, Ireland.http://www.worldpoultry.net/ *World Poultry 25: 4: 2009*

Stevenson, J.W. and Earle, I.P. (1956) Studies on parakeratosis in swine. *Journal of Animal Science* 15, 1036–1045.

Stocker, R, Keaney J. F Jr. (2004). Role of oxidative modifications in atherosclerosis. *Physiology Reviews*.84:1381–1478

Sturkie, P. D. (1970). Respiration in Birds. In: Duke's physiology of domestic animals (Swenson, M.J. Ed) 8th ed. Publishing Assoc. New York.

Sturkie, P. D. (1977). Blood Physical Properties, Formed Elements Haematology and Coagulation. In: Avian Physiology (5th Ed.) Sturkie, P. D. ed. Springriew Pub., London and New York.

Sunder, G., Shyam, A. K., Panda, N. C. S. Gopinath, S. V., Rama Rao, M. V., Raju, M. R., Reddy, L. N. and Vijay Kumar, C. (2008). Effects of Higher Levels of Zinc Supplementationon Performance, Mineral Availability, and Immune Competence in Broiler Chickens. *Journal of Applied Poultry Research* 17: 79–86.

Sutherland, G. B., Tarpan, I. L. and Campbell, D. H. (1958). Cold adapted animals 11: Changes in the circulating plasma proteins and formed elements of rabbit blood under various degrees of cold stress. *Journal of Applied Physiology* 121:362.

Suttle N. F**. (**2010). Mineral Nutrition of Livestock, 4th Edition, Library of Congress Cataloging-in-Publication Data ISBN 978-1-84593-472-9 (alk. paper) London, UK.

Swindle, M. M., Smith A. C., Laber, K. L., Goodrich, J. A. and Bingel, S. A. (2003). Biology and Medicine of Swine. In Laboratory Animal Medicine Management. J. D. Reuter and M. Suckow (Eds). *International Veterinary Information Service*, Ithaca, N.Y.

Szabo. G., Chavan, S., Mandrekar, P. Catalano, D. (1999). Acute alcoholic consumption attenuates IL-8 and MCP-1 induction in response to *ex vivo* stimulation. *Journal of Clinca Immunology* 19: 67-76.

Taiwo, V. O. and Ogunsanmi, A. O. (2003). Haematology, Plasma, whole blood and erythrocyte biochemical values of clinically health captive-reared grey duiker (Sylvicapra grimmia) and West African Dwarf sheep and Goats in Ibadan, Nigeria. Isreal Journal of Veterinary Medicine 58 (2 & 3) 57-61. www.isrvma.org/ journal.htm.

Tactacan, G. B (2001). Inorganic and proteinated trace minerals in broiler and layer diets. Agris 2001 - FAO of the United Nations.

Tewe, O. O. (1985). Protein metabolism in growing pigs feed corn or cassava peel based diets containing graded protein levels. *Research in Veterinary Science* 38: 259-263.

Teeter, R. G. and Dehyhim, F. D (1996). Vitamin withdrawal effects on performance, carcass composition, and tissue vitamin concentration of broilers exposed to various stress types. BASF Technical Symposium – Poultry Nutrition Up-date, Atlanta GA.

Thurston, R. J. and Bellier, H. V. (1972). Ultrastructure studies of turkey semen. *Poultry Science* 51:1879.

Thomas D. V. and Ravindran, V. (2010) Mineral retention in young broiler chicks fed diets based on wheat, sorghum or maize. *Asian - Australasian Journal of Animal Sciences* 23(1): 68-73

Todd, W.R., Elvehjem. C.A. and Hart, E.B. (1934) Zinc in the nutrition of the rat. *American Journalof Physiology* 107, 146–156.

Togun, V. A. and Egbunike, G. N. (2006). Seasonal variations in the sperm production characteristics of Zebu (White Fulani) cattle genitalia in the humid tropical environment. *Middle-East Journal of Science Resources* 1: In press.

Toro, G. and Ackerman, P. G. (1975) Practical Clinical Chemistry, little Brown and company Boston.

Trait, S. J. and Wright, A. J . (1990). Effecet of sugar biofibric and wheat bran fibre on iron and zinc absorption in rats. *British Journal of Nutri*tion 65: 546-552.

Treacher, A. L (1977). Dietary protein levels and blood composition in dietary cattle. *British Society of Animal science Occasional Publication* 1: 133- 142.

Trevor, P. (2001). Application of enzymatic analysis in medicine and industry.In Enzymes, Biochemistry, and chemical chemistry 1st ed. Harwood Publishing Ltd. West Succex, England. Pp. 340-344.

Tuncer, P.B., H. Kinet, N. Ozdogan and O.O. Demiral, 2006. Evaluation of some spermatological characteristics in Denizli cocks. *Journal of Faculty Veterinary Medicine University Erciyes* 3(1): 37-42.

Tumbleson, M. E., Hutchemson, D. P. and Van Burger, J. T. (1976). Serum Protein concentration and enzyme activity as a function of dye sex in miniature swine growth. *Journal of Animal Science* 40:53-68.

Udeh, I., Ugwu, S. O. C. and Ogagifo N. L. (2011). Predicting Semen Traits of Local and Exotic Cocks using Linear Body Measurements. *Asian Journal of Animal Sciences* 5 (4): 268-276.

Underwood, E. J. (1977.) Trace elements in human and animal nutrition. New York: Academic Press New York;

Underwood, E. J. (1981). The mineral nutrition of livestock. 2nd Agriculture Bureau, Slough, UK.

Underwood, E. J. and Suttle, N. F. (1999). The Mineral Nutrition of Livestock, 3rd Ed. CABI University of Illinois Urbana, Illinois. Published by the Salt Institute700 North.

Van Dujin, C. (1984). Relationship between spermatozoan numbers and fertility. *International Journal of Fertilility* 9:609.

Van Oeckel, M. J., Warrmant, N. and Bonkque, L. V. (1999). Measurement and prediction of pork colour. *Meat Science* 52: 347-354.

Vander Klis, J. D. and Kemme, A. D (2002). An appraisal of trace elements: inorganic and organic In: Poultry Feed-stuffs: Supply, Composition and Nutritive Value. J.M. MC-Nab and K.N.Boorman, CAB International, Wallingford,UK.

Vieira, S.L (2008) Chelated minerals for poultry, *Revista Brasileira de Ciência Avícola* 10:2 http://www.scielo.br/scielo.php? http://dx.doi.org/10.1590/S1516-635X2008000200001

W B E (1995). World Book Encyclopedia, Volumes 9,12,18 and 19. World Book Inc. Scott Fretzer Co., London.

Webb, K. E., Wong . E. A., Pan Y.X., Chen, H., Poole, C. A., Van, L. and. Klang, J. E (2005). The role of peptides in absorption pathways in Redefining Mineral Nutrition. J. A. Taylor-Pickard and L. A Tucker, Ed. Nottingham Univ. Press, Nottingham UK. pp197-225.

Wedekind, K. J. and Baker D. H. (1990). Zinc bioavailability in feed-grade sources of zinc. *Journal of Animal Nutrition* 68: 684-689

Wedekind, K. J., Lewis, A. J., Giesmann, M. A., Miller, P. S. (1994). Bioavailability of zinc from inorganic and organic sources for pigs fed corn-soybean meal diets. *Journal of Animal Science.* 72: 2681-2689.

Wedekind, K. J., S. Yu, and G. F. Combs. (2003). The selenium requirement of the puppy. *Journal of Animal Physiology And Animal Nutrtion* 88:340–347.

Wegger, I. and Palludan, B. (1978). In "Trace Element metabolism in man and Animals -3" (M Kirchgessner, ed.) Freisng-Weinen-Stephen, West Germany. pp 428.

Wellinghausen, N., Martin, M and Rink, L. (1997). Zinc inhibits IL-I dependent T cell stimulation. *European Journal of Immunology* 27: 2529–2535.

Wentworth, B. C. and Mellen, W. J. (1963). Egg production and fertility following various methods of insemination in Japanese quail. (Conternix Japonica). *Journal of Reproduction Fertilily* 6: 215.

Wettemann, R. P and Desjardins, C. (1979).Testicular function in boars exposed to elevated ambient temperature. *Biology of Reproduuction* 20: 235-241.

Wheater, P. R.., Daniel, V. G. and Burkit, H. G. (1987). Functional histology (2nd Ed) Churchill Livingstone, ELBC. pp 154-160

White, C. L. (1993). The zinc requirements of grazing ruminants. In: Robson, A.D. (Ed.) Zinc in soils and Plants: Developments in Plant and Soil Sciences. Kluwer Academic Publishers, London. 55:197.

Wikipedia (2002). The Liver Tests, Wikipedia, the free encyclopedia. en.m.Wikipedia.org/wiki/Liver_function. Assessed on 3rd March, 2014.

Wilde, D. (2006). Influence of macro and micro minerals in the peri-parturient period on fertility in dairy cattle. *Animal Reproduction Science* 96:240-249.

Wilson, P. N. and Medd, R. K. (1978). Blood profile as a guide to nutritional status in animals. *British Society of Animal Production* 1:155-162.

Wintrobe, M. M. (1983). Microscopic examination of blood. *American Journal of Medical Science* 1985:58-59.

Wolders, H. (1991). Overview of in vitro methods for evaluation of Semen Quality. *Reproduction of Domestic Animal* 1: 145-164

Woelders, H. (1981). Overview of invitro methods for evaluation of semen quality, *Reproduction of Domestic Animal Suppl* 145:1641

Zago M. P, Oteiza P. I. (2001). The antioxidant properties of zinc: interactions with iron and antioxidants. *Free Radical Biology and Medicine* 31:266 –274.

Zemjanis, R., (1970). Collection and evaluation of semen.In: Diagnostic and Therapeutic Techniques in Animal Reproduction, 2nd Edn. Published by Williams and Wilkins Company, Baltimore, Maryland. pp 139-156

Zhou W., Park, S., Liu, G., Miller, D. P., Wang, L. I., Pothier, L., Wain, J. C., Lynch, T. J., Giovannucci, E. and Christiani, D. C. (2005) National Center for Biotechnology Information, U.S.National Library of Medicine 8600 Rockville Pike, Bethesda xMD, 20894 USA Epidemiology. 16(6):772-779.

Zinpro corporation, ZC (2002). Internal research. Zinpro Corporation, Fourteen trial summary on feeding complexed zinc and manganese to broilers. Assessed on 13rd May, 2013.