EFFECTS OF VARYING DIETARY LEVELS OF NANO SELENIUM ON GROWTH PERFORMANCE, GUT MORPHOLOGY, HAEMATOLOGY, IMMUNITY AND CARCASS PARAMETERS OF BROILER CHICKENS

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ABSTRACT

This study determined the effects of supplementation of dietary nano Selenium on growth performance, meat quality, haematological, immunity, serum biochemistry and gut morphology of broiler chickens. A total of 200 1-day old *Arbor acre* broiler birds were distributed into five treatments using a completely randomized design. Each treatment had four replicates with 10 birds per replicate. Treatment 1 served as the control which had 0 level of nano Se and tagged $NSe_{0.00}$. Treatments 2,3,4 and 5 had 0.10, 0.15, 0.20 and 0.25 mg/kg of nano Se and were tagged $NSe_{0.10}$, $NSe_{0.15}$, $NSe_{0.20}$ and $NSe_{0.25}$ respectively. Data on growth performance, meat quality, haematological, blood serum, immunity and gut morphology were taken. They were analysed using one way analysis of variance and means separated using Least Significant Differences (LSD) where significant. Results of the study showed that the supplementation of dietary nano Se at 0.25 mg/kg improved ($P<0.05$) the Body Weight Gain, BWG (990.32 g) and Feed Conversion Ratio, FCR (1.93) compared to the control (684.73 g) and (2.63) respectively at the starter phase of the experiment. Birds fed 0.25 mg/kg dietary nano Se supplemented diets had higher ($P<0.05$) BWG (1339.78 g) and better FCR (1.94) compared to birds fed the basal diet having BWG (958.01 g) and FCR (2.60) respectively at the grower phase of the study. Similarly, birds fed diets supplemented with 0.25 mg/kg dietary nano Se had improved ($P<0.05$) BWG (2262.84 g) as compared to the control (1881.50 g) at 0 – 7 weeks of the study. Supplementation of dietary nano Se at 0.25 mg/kg have a better ($P<0.05$) dressing percentage (74.87 %) than the control (57.42 %). Supplementation of nano Se at 0.20 mg/kg have a better ($P<0.05$) Dry Matter, DM values at both starter (89.02) and finisher (89.65) phases compared to birds fed the control diet (79.21) and (81.51) respectively. Furthermore, birds fed $NSe_{0.20}$ supplemented diet have a better ($P<0.05$) cooking loss value (2.14) compared to birds fed the basal diet (6.05). Similarly, birds fed $NSe_{0.20}$ had their meat more acceptable (2.50) compared to the control group (1.00). Furthermore, better ($P<0.05$) WHC values at both 0 (4.02) and 24 (2.41) hours after slaughter were recorded for birds fed 0.25 mg/kg nano Se supplemented diets compared to those recorded for birds fed the basal diets at 0 (0.42) and 24 (0.23) hours after slaughter. The RBC (7.30) and WBC (5.35) of birds fed 0.20 mg/kg dietary nano Se were improved ($P<0.05$) compared to those birds fed the basal diet (5.95) and (4.05) respectively. The urea (4.37) of birds fed dietary 0.25 mg/kg nano Se were improved ($P<0.05$) compared to the control (6.30) while the birds fed 0.20 mg/kg nano Se supplemented diets had improved ($P<0.05$) Ig A (1.82) as compared to those fed the control diet (2.62). Better ($P<0.05$) crypt depth was recorded for birds fed 0.25 mg/kg nano Se (74.45) compared to birds fed the basal diet diet (64.29). It can be concluded that the supplementation of nano Se at 0.25 mg/kg in the diets of *Arbor acre* broiler birds could improve their growth performance, meat quality, haematological, immunity and gut morphology.

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Glossary of Abbreviations

ANOVA: Analysis of Variance

Africa Sustainable Livestock

AOAC: Association of Official Analytical Chemists

BWG: Body Weight Gain

CRD: Completely Randomised Design

ELISA: Enzyme – linked immunosorbent assay

EU: European Union

FAO: Food and Agricultural Organisation

FCR: Feed Conversion Ratio

FDA: Food and Drug Administration

FI: Feed Intake

GIT: Gastrointestinal tract

HCW: Hot Carcass Weight

HI: Haemaglutination Inhibition

Ig: Immunoglobulin

LSD: Least Significant Difference

MCS: Meat Cooking Shrinkage

NCD: Newcastle Disease

NP: Nano particles

NRC: National Research Council

NSe: Nano Selenium

SAS: Statistical Analysis System

SCENIHR: Scientific Committee on Emerging and Newly Identified Health Risks

Se: Selenium

USDA: United States Department of Agriculture

WHC: Water Holding Capacity

OIE: Office International des Epizooties

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Poultry are domesticated birds raised for meat or egg production. Domestic fowls, geese, turkeys, ducks, guinea fowls, and quails are some examples of poultry (Aboki *et al*., 2013). Regardless of culture or religion, it is undoubtedly one of the most common sources of animal protein consumed worldwide. Broilers in this case are a type of domestic bird and common breeds of broiler chickens include Cochin, Cornish, and Sussex (Oluyemi and Roberts, 2006).

According to the Food and Agricultural Organization (FAO, 2018), Nigeria's chicken population is estimated to be over 180 million. Around 80 million of these chickens are raised in extensive systems, 60 million in semi-intensive systems, and the remaining 40 million are raised in intensive systems (Africa Sustainable Livestock, ASL 2050, 2018). According to SAHEL (2015), Nigeria has been reported to be Africa's second highest chicken producer after South Africa, producing approximately 650,000 tonnes of eggs and 300,000 tonnes of poultry meat in 2013 (FAO Statistics, 2018). However, this figure falls short of the expected level of animal protein consumption.

Researchers have fortified poultry meat with essential minerals like selenium in response to consumer demand for safer and increased animal protein intake. Selenium (Se) is a micro mineral that is required for both human and animal health. This element is well-known for its role in chicken growth (Yoon *et al*., 2007; Wang and Xu 2008), immune competence (Cai *et al*., 2012; Liao *et al*., 2012), feather formation (Edens *et al*., 2001; Peric *et al*., 2009), and reproduction (Leeson *et al*., 2008). Selenium is a trace mineral that has captured the interest of nutritionists due to its numerous biological activities that

influence growth and health (Yoon *et al*., 2007; Surai *et al*., 2018). It has a significant impact on egg quality (Chantiratikul *et al*., 2008), meat quality (Li *et al*., 2018), and antioxidant activity (Jing *et al*., 2015).

Supplementing Se in the diet of broiler chickens leads to increased antibody titre to Newcastle disease (ND) (Hegazy and Adachi, 2000). Selenium is incorporated into selenoproteins, such as the amino acid selenocysteine, which influences immunological response (Hoffman, 2007). Some minerals have been recommended as antioxidants which helps to manage oxidative stress observed in broiler chickens among which include Selenium, Zinc, Manganese, and Copper (Willcox *et al*., 2004). Se and vitamin E have been shown to play important roles as antioxidants in avian reproduction, and supplementing one or both at an optimum level appears to be necessary (Surai *et al*., 2016).

Despite the important roles that this mineral plays, it is still one of the most deficient minerals in chicken diets, resulting in low productivity (Chrastinova *et al*., 2016). As a result of its deficiency, cases of exudative diathesis and pancreatic degeneration appear to reoccur (Toghyani *et al*., 2008) as such, there is a need to supplement Se in chicken feed in other acceptable forms, such as nano size form.

Nanoparticles are small core particles (1-100 nm) that function as a single unit in terms of characteristics (Nour and Yunus, 2010; Thulasi *et al*., 2013). They have been reported to have a larger potential than their conventional sources of inorganic Se (primarily selenite) and organic Se (mostly selenomethionine), resulting in a reduction in the quantity needed (Sri Sindhura *et al*., 2014).

With the recent development of nano technology, nanoselenium (nano Se) has gotten a lot of interest recently as a result of its unique properties, such as high catalytic efficiency,

increased surface area, low toxicity, high surface activity, and strong adsorbing capacity (Wang *et al*., 2007; Zhang *et al*., 2008). Furthermore, Zhang *et al*. (2008) found that nano Se regulates selenoenzymes as effectively as selenite and se-methylselenocysteine, but with a significantly lower toxicity.

Several research have been conducted to discover the main variations in the diets of chickens from various sources of Se (organic, inorganic, and nano). There are variances in this element's absorption, accumulation, and distribution in poultry tissues, which mostly depends on the dietary Se source (Surai *et al*., 2016). Similarly, the expression of selenoproteins is largely regulated by the level of dietary Se (Tarze *et al*., 2007).

Chemical reactivity has increased significantly when broiler chickens were supplemented with nano Se (Suchy *et al*., 2014). *Cai et al*. (2012) investigated the effects of nano Se on meat yield, meat quality, oxidation resistance, immune functions, and levels in broilers, concluding that there were significant effects on glutathione peroxidase (GSH-Px) activity, immunoglobulin M (IgM) serum levels, and free radical inhibition. GSH-Px is an enzyme that converts hydrogen peroxide, which is harmful and carcinogenic, into oxygen and water. The enzyme can be activated with a modest quantity of Se (selenocysteine). Its primary role is to remove excess peroxide and hydrogen peroxide from fatty acids produced by lipid exudation (De Almeina *et al*., 2012).

Replacing sodium selenite or at least a portion of it, in poultry diets with alternative forms such as nano has been shown to improve fertility and hatchability of birds (Surai *et al*., 2006). Inorganic salts such as sodium selenite are the most common ways to add Se to poultry diets, although their bioavailability and toxicity levels are constraints (Karadas *et al*., 2005). As a result of this, researchers must identify new Se forms with dramatically reduced toxicity, enhanced bioavailability, and efficacy. As a result, nano Se has received

a lot of attention, and research on comparative toxicity and efficacy have revealed that its toxicity is lower and its bioavailability is higher (Li *et al*., 2012).

1.2 Statement of the Research Problem

Due to the great improvements recorded in genetics, modern commercial poultry birds are said to have a very fast growth rate and good feed efficiency. However, there is a significant drawback to such improvements in performance because birds are commonly susceptible to conditions such as oxidative and heat stress (Willcox *et al*., 2004). As a result, studies have been conducted to discover the optimal Se supplementation in broiler diets in order to provide the best possible productivity.

According to research findings, it has been proven that dietary Se is essential for normal broiler growth, and supplementing it in nano form improves its utilization (Muhammad, 2017). When minerals are supplied in excess to broiler chickens, it results to the pollution of the environment as they are expelled through their faeces (Rohner *et al*., 2007). Deficiency of Se causes exudative diathesis and pancreatic atrophy (Toghyani *et al*., 2008). This has prompted scientists to consider alternative methods of supplying these minerals to birds that will result in safe animal products, improved use, and minimum contamination. However, there is a paucity of data on the optimal amount of nano form of Se required to achieve optimal broiler performance.

The optimal dosage of Se in nano form has not been fully explored. The dietary effects of nano Se on blood serum and gut morphology have not been fully established. This study aimed at investigating the effects of nano Se on growth performance, carcass, haematological, immunological and guts morphological parameters of broilers.

1.3 Justification for the Study

Nano Se supplementation in broiler diets increased growth performance, immunological response, and carcass characteristics of broiler chickens reared in temperate climates,

according to several studies (Salim *et al*., 2015; Dalia *et al*., 2017; Ahmadi *et al*., 2018). This is because it possesses fine particles, a greater surface area, high bioavailability, high catalytic efficiency, low toxicity, and a strong adsorbing ability, all of which contribute to its improved utilization.

For feed safety, the maximum quantity of Se to be supplemented in diets has been set at 0.5 mg/kg, based on recommendations from the European Union (2004). In addition, the NRC (1994) recommended a Se concentration of 0.3 ppm, and Zhou and Wang (2011) found that supplementing chicken feed with 0.30 mg/kg of nano Se resulted in an effective boost in growth performance. It has also been established that Se's bioavailability is largely correlated to its physical form (Ahmadi *et al*., 2018).

There is a shortage of information about the impact of Se deficit on impaired humoral immunity in poultry and pigs. Only a few studies have found a beneficial effect of dietary Se supplementation in chickens on the production of particular antibodies by Infectious Bursal Disease (IBD) virus vaccines (Arshad *et al*., 2005; Shekaro *et al*., 2012). When organic Se of various concentrations (0, 100, 200, 300, or 400 µg/kg diet) was supplied in a bid to assess the role of Se on broiler immunity, Rao *et al*. (2013) found no effects on the production of antibodies specific for vaccines against Newcastle disease.

There is widespread attention on nano Se since it exhibits novel characteristics like high catalytic efficiency, larger surface area, low toxicity, high surface activity and strong adsorbing ability (Wang *et al*., 2007; Zhang *et al*., 2008). However, both the recommendations made by NRC and European Union are largely based on research findings conducted in the temperate world. Such requirements may not be optimum for broilers raised in the tropical regions like Nigeria. It is therefore imperative to conduct studies to evaluate the optimum requirements of nano Se in other ecological zones.

1.4 Aim and Objectives of the Study

This research aimed at investigating the effects of varying levels of dietary nano Se on growth performance, haematological, blood serum, immunity, carcass and gut morphological parameters of broiler chickens. The objectives of the study were to determine the effects of supplementing dietary nano Se on:

i. growth performance of broiler birds;

ii. haematological and blood serum properties of broiler birds;

iii. immune response of broiler birds;

iv. carcass characteristics of broiler birds; and

v. gut morphology of broiler birds.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Concept of Nanotechnology in Poultry Production

Nanotechnology was coined from the Latin term nanus, which means dwarf. Norio Taniguchi was the first to deploy this technique in 1974, however the concept was established by a renowned scientist named Richard Feynman in 1959 to reduce particle size to a few nanometers (Marappan *et al*., 2017; Damian and Konrad, 2018). The Food and Drug Administration (FDA, USA) in 2006 defined nanomaterials as particles smaller than micrometric scales that display certain qualities (Miller and Senjen, 2008). For the first time, the application of this field in agriculture and the food industry was discussed in the United States Department of Agriculture (USDA) action plan, which was released in September 2003 (Joseph and Morrison, 2006).

Nanoparticles can be classified into three types based on their size, according to the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR, 2010). Big particles have a diameter higher than 500 nm, medium particles have a diameter of 100 nm to 500 nm, and ultrafine particles have a diameter of less than 100 nm.

The application of this unique discovery has also been extended to the care of livestock (Chen and Yada, 2011), with nano minerals being the most significant application in this area. Nano minerals have a molecule size of 1-100 nm. At high temperatures and pressures, a percentage of these nano minerals have been found to be stable (Stoimenov *et al*., 2002). According to Vinus and Sheoran (2017), nanoparticles will most likely be distinguishable from their conventional structures due to their outrageous small size and outstanding physical properties. Nanotechnology can also help to shorten the time it takes for birds to start producing meat and eggs.

There has been a recent increase in consumer knowledge and interest in animal-derived protein. This can be attributed to the fact that buyers are looking for food with prohealth benefits, a longer shelf life, and high sensory qualities. The use of nano minerals in animal nutrition has been prompted by a series of beneficial outcomes in increasing the content and quality of animal-derived products (Damian and Konrad, 2018).

Nano-feed additives, according to El-Sabry *et al*. (2018), could aid in boosting feed efficiency, lowering feed costs, and raising the quantity and quality of animal products. Due to their positive influence in increasing productivity and liveability, nano feed additives and novel detoxifying nano minerals are projected to bring added value in feeding practices in intensive poultry and ruminant production.

According to current worldwide predictions, it has been asserted that chicken meat will have the highest level of production and consumption by 2025, surpassing beef, pork, veal, and mutton (OECD/FAO, 2016). This forecast necessitated the development and transformation of nanotechnology as a technical advancement that will expand and transform the agrifood sector, with the ability to enhance global food production while also improving the nutritional value, quality, and safety of food (Handford *et al*., 2014; Peters *et al*., 2016).

Despite the widespread acceptance of nanotechnology, a number of concerns have been expressed about the safety and regulation of nano materials due to the lack of clarity about their negative impacts on consumers and the environment. Nonetheless, over the last decade, research into nano-enabled technologies has exploded, and numerous businesses involved in the manufacture of novel nano-sized materials have discovered applications for more efficient and safer chicken production (Duncan, 2011).

Some notable countries, such as the United States, the European Union, China, Sweden, Denmark and Germany, have prioritized research and development that produce nano materials (Zhao *et al*., 2008; de Wit, 2009; Kastenhofer, 2011). According to Zhao *et al*. (2008) and de Wit (2009), nano materials are a field of research with significant socioeconomic potential for the development of medications, pesticides, and fertilizers, as well as the mitigation of environmental problems. Similarly, Roco *et al*. (2010) reported that in 2008, more than $15 billion was spent on over 400,000 studies in nanotechnology research and development around the world.

2.2 Methods of Preparing Nanoparticles

Nanotechnology is a relatively new and rapidly developing technology with a wide range of applications. It entails the synthesis and application of materials with dimensions ranging from 1-100 nm. Nanoparticles (NP) have been created by a number of physiochemical methods. Biogenic reduction of metal precursors to produce equivalent NPs has been shown to be environmentally benign, less expensive, and free of chemical contaminants for medical and biological applications where NP purity is important (Hussain *et al*., 2016). Due to the wide range of its usage, there has been a growth in demand, leading to the development of more sensitive and effective methods of synthesizing chosen NPs. The primary goal of synthesizing these NPs is to improve particle size, purity, quality, quantity, and morphology (Hahn, 1997).

Physical, chemical, and biological methods can all be used to synthesize nano minerals. The biological method, which is also eco-friendly, has been demonstrated to be the safest to use and may be efficiently exploited without further experiment on the residual effect (Rajendran, 2013; Sri-Sindhura *et al*., 2014).

Biological synthesis has recently grown in popularity as a viable alternative to traditional NP synthesis methods. Actinomycetes (Ahmad *et al*., 2003; Sastry *et al*., 2003), viruses

(Lee *et al*., 2002), bacteria (Joerger *et al*., 2001; Nair and Pradeep, 2002), plants (Kumar *et al*., 2010), yeast (Kowshik *et al*., 2003), and fungi (Kowshik *et al*., 2003) are examples of unicellular and multicellular entities (Kuber and Souza, 2006). Plants, fungus, viruses, bacteria, and algae have all been employed extensively in the production of low-cost, energy-efficient, and non-toxic metallic nanoparticles in recent years (Kaushik *et al*., 2010). Silver, gold, cadmium, selenium, titanium, palladium, and barium titanate have all been successfully synthesized using biological methods (Sharma *et al*., 2007; Narayanan and Sakthive, 2010; Philip, 2011) using various plant materials such as *Avena sativa*, alfalfa, lemon grass, *Azadirachta indica*, *Sesbania drummondii*, latex of Jathropha cutcas, and papay (Shankar *et al*., 2004).

There are several ways for synthesizing NPs, but they can be grouped into two categories as indicated in Figure 2.1: bottom-up and top-down approaches (Wang and Xia, 2004; Ibrahim *et al*., 2019).

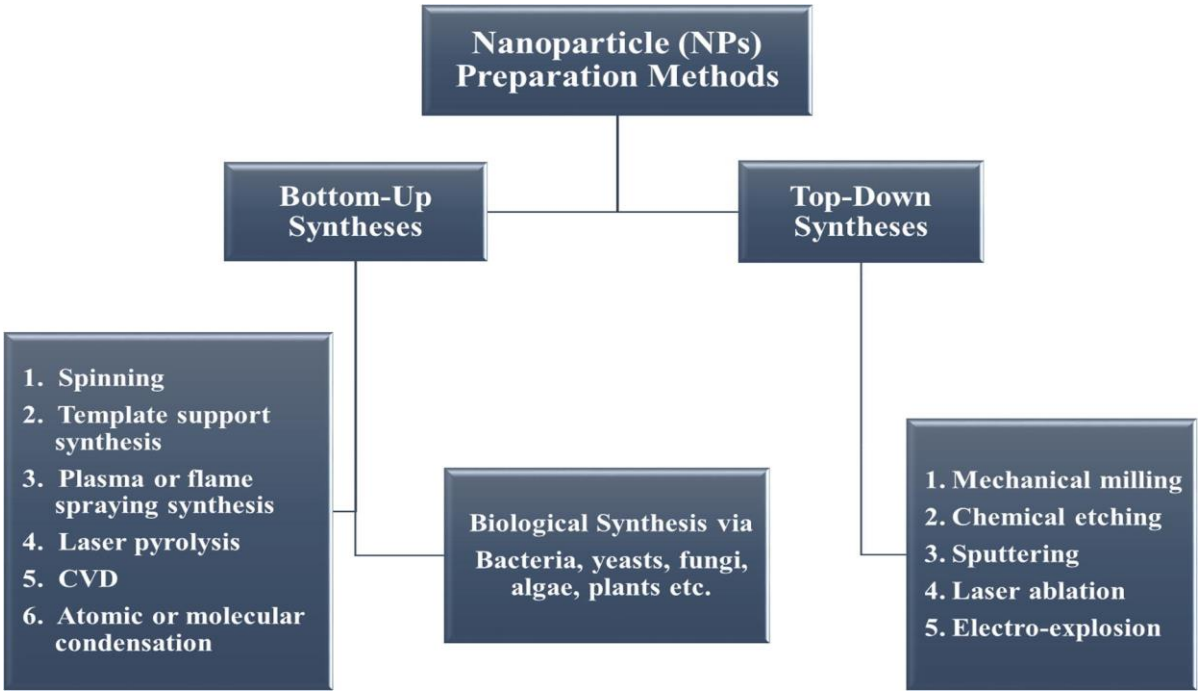

Fig. 2.1: Typical synthetic methods for NPs for the top-down and bottom-up approaches

Source: Wang and Xia, (2004)

According to Monaliben *et al*. (2015), during biological synthesis, a variety of regulating factors play a role in the nucleation and subsequent production of stabilized metallic NPs. Temperature, pH, reactant concentrations, and reaction time are all parameters to consider. When NPs are synthesized using biological entities that operate as biological factories, they provide a safe, non-toxic, and environmentally friendly method of synthesizing NPs with a wide range of shapes, sizes, compositions, and physicochemical properties (Mohanpuria *et al*., 2008). When compared to other biological systems, biosynthesis of NPs using plants or plant-based extracts is generally safe, have relatively short production times and a cheaper cultivation cost compared to other biological systems (Mittal *et al*., 2013).

2.3 Effects of feeding nano selenium on growth performance of broiler birds

Salim *et al*. (2015) investigated the effects of feeding five Se sources: sodium selenite as an inorganic form, selenomethionine as an organic form, Zinc-L-selenomethionine as a more recent organic form, powdered nano Se, and liquid nano Se. The use of either organic or nano form of Se or raising the level of inclusion of Se from 0.15-0.30 ppm in broiler feeds resulted in a significant improvement in growth performance and Se concentration in liver and thigh tissues, according to the study's findings. Ibrahim *et al*. (2019) used 450 Ross broilers to compare the effects of different Se forms (Sodium selenite; SeS, selenomethionine; Met-Se or nano Se) and levels on growth performance, Se retention, antioxidative potential of fresh and frozen meat, and genes associated to oxidative stress. The birds were fed diets containing 0.3, 0.45, and 0.6 mg Se/kg in the form of SeS, Met-Se or nano Se. Body weight gain was considerably increased ($P<0.05$) with Met-Se or nano Se supplementation, according to the results of the experiment. When compared to the group fed the same amount of SeS, the feed conversion ratio was also improved at 0.45 and 0.6 mg/kg.

Hamzekolaei *et al*. (2018) fed four diets (control, basal diet + 500 mg/kg vit. C, basal diet + 0.3 mg/kg nano Se and basal diet + 0.3 mg/kg organic Se) to the experimental birds. The study's findings revealed that the nano Se group's FCR was quantitatively better, but

there was no statistical difference (P>0.05). Similarly, the relative carcass weight was quantitatively higher in the vitamin C and nano Se groups than in the other groups.

Zhou and Wang (2011) investigated the effects of nano Se on Guangxi Yellow chickens at varying doses of 0.10, 0.30, and 0.50 ppm of the test element. The results showed that the groups supplemented with nano selenium had better final body weight, daily body weight gain, FCR, and survival rate when compared to the control group. Similarly, Ahmadi *et al*. (2018), in a study on varying levels of supplemental nano Se at 0.00 mg/kg, 0.10 mg/kg, 0.20 mg/kg 0.40 mg /kg and 0.5 mg/kg reported that treatments supplemented with nano Se improved weight gain and FCR significantly in the starter (1st-21st day), grower (22nd-42nd day), and whole (1st-42nd day) when compared to the control group, which had no test mineral. Birds fed 0.30 mg/kg, on the other hand, showed the best growth results.

Cai *et al*. (2012) fed diets at supplemental levels of 0.00, 0.30, 0.50, 1.00, or 1.20 mg/kg of nano Se and reported no significant differences in performance (P>0.05) as a result of the nano Se supplementation. Wang (2009) reported a positive finding on growth performance of avian broilers fed a supplemental nano Se at 0.2 and 0.5 ppm. When compared to the control group and those fed sodium selenite, Mohapatra *et al*. (2014) found that layer birds supplemented with different doses (0.075, 0.15, 0.3, 0.6 ppm) of nano Se had significantly higher body weight, breast muscle, Se content in liver, and feathers.

The effects of nano Se on growth performance of broiler chickens reared under thermoneutral (22 ± 1 ^{0}C) or high ambient temperature (35 ± 1 ^{0}C) conditions using 36 broiler chicks at 15-d old in a 3 x 2 factorial design was examined by El-Deep *et al*. (2016). The results showed that high ambient temperature depressed body weight gain,

FCR, breast muscle weight, feed intake and abdominal fat weight. It was, however, observed that these negative effects were clearly alleviated in nano Se supplemented treatments unlike the treatments that had sodium selenite.

2.4 Effects of feeding nano Se on meat quality characteristics.

In life science, there are some differences in what is referred to as "meat." It is believed to be a tissue made up primarily of muscular flesh, which is about 20% protein, 75% water, 1-10% fat, and 1% glycogen (Listrat *et al*., 2016). Meat is a good source of protein as it contains various minerals, vitamins, and fatty acids, and thus plays a vital part in human life, religion, culture, and, most importantly, nutrition (Sredinacka *et al*., 2016). With the rapid advancement of modern animal production and the growing knowledge of customers, meat quality has become increasingly important in terms of nutritional and organoleptic properties (Grunert et al., 2004). Properties such as its size, appearance, fatty acid profile, freshness, cholesterol and mineral contents continue to be of more interest to the consumers (Wang *et al*., 2014).

Cai *et al*. (2012) investigated the effects of nano Se administered to the birds at 0.0, 0.3, 0.5, 1.0 and 2.0 mg/kg of corn-soybean meal based diets. The results of the study showed that supplementing with nano Se yielded no significant differences ($P>0.05$) in meat colour, performance or immune organ index (thymus, bursa and spleen).

Salim *et al*. (2015) reported that giblet %, carcass abdominal fat % and malnodialdehyde (MDA) contents in thigh muscle were not affected due to Se sources or levels fed to broiler birds. However, the Se concentration in liver was greater than that of the thigh muscles. Ibrahim *et al*. (2019) fed broiler birds diets supplemented with 0.3, 0.45 and 0.6 mgSe/kg as sodium selenite; SeS, selenomethionine and nano Se and showed that group fed supplemental nano Se retained the mineral more in the breast muscle ($P<0.05$) compared to the SeS group, especially, at a level of 0.6 mg/kg.

The effect of this test mineral on carcass quality was also studied by Ahmadi *et al*. (2018) in which varying levels of nano Se was supplemented in the feed of broilers at the rate of 0.00 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg and 0.5 mg/kg. The results of the experiment suggested that both breast (0.3 mg/kg) and drumstick (0.2 mg/kg) percentages had higher weight values in the nano Se supplemented group than the control while the reverse was recorded for the abdominal fat percentage.

2.5 Effects of Feeding Selenium on Immune Functions of Poultry.

Disease prevention and control is one of the most significant issues encountered by poultry farmers, as diseases can easily spread from infected to healthy animals (El Sabry *et al*., 2012). The use of vaccinations and medications to treat and prevent these diseases in broiler chickens has a number of drawbacks, including the virulent reversion of pathogenic strains against live vaccines and the danger of instability (Lowenthal *et al*., 2005; Peek *et al*., 2008). Furthermore, the uncontrolled use of antibiotics and chemicals in poultry has resulted in the creation of resistant strains of genetically modified pathogens, increasing environmental contamination and residual contents in meat and eggs. Consumers face a significant public health risk as a result of the transmission of antibiotic resistance from animal products to humans, as they are the end users of the products (Feng *et al*., 2009). In light of the foregoing, animal nutritionists were left with no other option but to investigate alternative antibiotics and vaccines that could aid broiler chickens' immune systems. Minerals such as selenium, zinc, and chromium have recorded a substantial impact in this regard (Powell *et al*., 2000; Rajendran, 2013; Sahoo *et al*., 2014).

Some studies have found that supplementing Se in amounts exceeding nutritional requirements has a favorable effect on necrotic enteritis, coccidiosis, and avian pathogenic E. coli resistance in chicken, since feeding this mineral has considerably

boosted resistance to these diseases (Larsen *et al*., 1997; Mahmoud and Edens, 2005; Wunderlich *et al*., 2014; Xu *et al*., 2015). According to Surai and Dvorska (2002) who reported that dietary Se is essential for the activity of virtually all arms of the immune system. aminSimilarly, Hoffman (2007) suggested that Se influences immunological response through incorporating it into selenoproteins such as amino acid selenocysteine, and that there are various mechanisms by which these selenoproteins influence immunity (Huang *et al*., 2012).

When Se is integrated into selenoproteins, it leads to the mineral intake as it regulates oxidative stress, redox, and other essential physiological processes in almost all cells and tissue types, including those involved in innate and adaptive immunity (Dalgaard *et al*., 2018).

Macrophages are key innate immune system professional scavenger cells (Dalgaard *et al*., 2018). Studies have shown that a diet deficient in vitamin E and selenium has a deleterious influence on macrophage numbers and phagocytic potential in chickens (Dietert *et al*., 1990).

According to Koski and Marilyn (2003), Se is required in the diet of tropical birds in order to sustain appropriate immune system growth and function. This is comparable to the findings of Zhang *et al*. (2012), who found that adding Se in broiler chicken diets boosted immune parameters. Se shortage also lowers antibody formation, cytokine synthesis, cell-mediated cytotoxicity, and lymphocyte proliferation (Powell *et al*., 2000).

Peric *et al*. (2009) reported that immune system cells release more free radicals, which kill pathogenic agents. Selenium is one of the most important minerals for preventing arachidonic acid peroxidation and protecting immune system cells and tissues from free radical damage (Canogullari *et al*., 2010; Ahmad *et al*., 2014). It also plays a key role in

the formation of peroxidase glutathione, which improves the immune system by boosting the production of white blood cells and thymus activity (Invernizzi *et al*., 2013).

Savaram *et al*. (2013) studied the effects of supplementing various concentrations (0, 100, 200, 300 and 400 mg/kg diet) of organic Se on immune response of commercial chickens. Results obtained indicated that ratios between heterophyls, lymphocytes, relative weight of lymphoid organs (bursa, spleen and thymus) and antibody production to Newcastle disease vaccination were not affected by supplementation of Se in the diets of broiler birds. However, there was a linear increase in the cell mediated immunity (lymphocyte proliferation ratio). The authors further reported that both antioxidant status and lymphocyte proliferation were not influenced by the supplementation of Se. This is in line with the findings of Cai *et al*. (2012) who reported that there were no significant differences in the immune organ index (thymus, bursa and spleen) due to supplementation of nano Se.

Shabani *et al*. (2019) studied the effects of three Se sources on performance and characteristics of immune system of broiler chickens using 500 Ross 308 strain in a completely randomised design. The experimental birds were administered diets supplemented with 0.5, 0.8 and 1.2 mg nano Se + basal diet, 0.5, 0.8 and 1.2 mg selenomethionine + basal diet and 65, 80 and 100 mg vitamin E + Se + basal diet. Group treated with nano Se produced a significant difference in the antibodies that were produced against Newcastle and Influenza. The total immunoglobulin titre and Immunoglobulin G produced were significantly increased compared to those in the other treatments including control. In a related study of the effects of nano Se on immune response of broiler birds carried out by Fuxiangl *et al*. (2008), the authors used 225 1-day old avian broiler chickens. They were fed diets supplemented with 0, 0.15, 0.3, 0.6 and 1.2 mg/kg of nano Se. The results of the study indicated that there was a significant

increase in the serum antibody titres of the Newcastle disease virus and the immune organ index at the range of 0.15-1.2 mg/kg of nano Se at 14 and 28 days. Furthermore, a significant increase in the transformation rates of the peripheral T-lymphocyte at the range of 0.6 - 1.2 mg/kg at 14, 28 and 42 days were observed. Therefore, the researchers concluded that the immune functions of avian broiler chickens can be promoted using nano Se at the level of 0.6 - 1.2 mg/kg.

2.6 Effects of Feeding Selenium on Gut Morphology of Poultry Birds

The study of the guts of broiler birds, which comprise the intestine and the caecum, is known as gut morphology. It has been demonstrated that changes in intestinal processes such as villi shape and microbial population are strongly linked to commercial broiler chickens' efficient feed utilization. Furthermore, the level of equilibrium between the host, intestinal microbiota, intestinal microscopic characteristics, and feed influence the gut health of chickens (Biasato *et al*., 2018).

The small intestine and peripheral organs are the major organs of the gastrointestinal tract (GIT) responsible for digestion and absorption. The physical and chemical processes that take place in the small intestine are crucial for food digestion and absorption (Adamnezhad and Jamshid, 2018). However, due to their rapid growth rate, the small intestine, villi, and depth of Lieberkuhn crypts in domestic fowls are finished during their early stages of life (Lilburn and Loeffler, 2015). Similarly, traditional free range chickens have a greater diversity of intestinal bacteria than intensively maintained birds (Cui *et al*., 2017; Chen *et al*., 2018).

According to Ensign *et al*. (2012), oral administration of nanoparticles remains the most appropriate and cost-effective method, though the presence of absorption barriers in the digestive tract (mucus covering the intestinal mucosa and the intestinal mucosa) can make this method slightly difficult because the barrier must be overcome.

According to studies, around a quarter of the gut microbiome has the potential to produce selenoproteins, and the availability of Se in the microbiological medium influences their expression (Kasaikina *et al*., 2011). As a result, these proteins serve an important role in both bacteria and mammalian hosts, where they are required for a variety of biological processes (Labunskyy *et al*., 2014).

As an antioxidant, Kasaikina *et al*. (2011) found that Se, in addition to increasing the composition and rate of intestinal microbiota, can improve the morphology of the gut (duodenum, ileum, jejunum, and colon). Se deficiency has been shown to have negative consequences for poultry. While Liu *et al*. (2016) suggested that Se deficiency can lead to intestinal mucosal inflammation, Wang *et al*. (2012) found that Se deficiency causes degranulation of mast cells in the jejunum of chickens with vacoulisation and granulation of epithelial cells.

The effects of variable doses of nano Se supplementation on the GIT of chickens using 180 male Ross chicks were studied by Ahmadi *et al*. (2019). The test element was varied from 0.1-0.5 mg/kg. Results obtained showed a decrease in the weights of small and large bowels with simultaneous increase in their lengths.

A study to determine the effects of nano Se and organic Se in comparison with vitamin C on growth performance, small intestine morphology and lipogenesis in broiler chickens was carried out by Hamzekolaei *et al*. (2018). A total of 192 one-day old Ross 308 broiler chickens divided into four treatment groups administered basal diet, vit. C (basal diet + 500 mg/kg vit. C), nano Se (basal diet + 0.3 mg/kg nano Se) and organic Se (basal diet + 0.3 mg/kg organic Se) were used for the experiment. Results obtained showed that the morphological parameters of the lieberkuhn gland depth, intestine, villi height and surface of duodenum were improved in nano Se group compared to others and was

significantly different with the control group in all of the parameters. Investigations of the effects of different levels of Vit. E and Se on growth performance and small intestine morphometry in 405 mixed sex Japanese Quails carried out by Adamnezhad and Jamshid (2018). The authors adopted a 2 x 3 factorial design arrangement using vit. E (0, 150 and 300 mg/kg) and sodium selenium (0, 0.2 and 0.4 mg/kg). Results obtained suggested that supplementing quail diet with vit. E and Se produced a significant increase in villi height and crypt depth in various sections of small intestine on day 35.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted at the Old Poultry Research Unit of the Department of Animal Production, School of Agriculture and Agricultural Technology, Federal University of Technology, Bosso Campus, Bosso Local Government, Niger State, Nigeria. FUT Minna Bosso Campus is located between Latitudes 9º39'3.82''N to 9º39'25.90"N and Longitude 6º31'27.65"E to 6º31'27.65"E (Odekunle *et al*., 2018). It has an average annual temperature of 27.5ºC and an average rainfall of 1229mm. The entire landscape of the state is covered by the Southern Guinea Savannah vegetation (Weather spark, 2019).

3.2 Experimental Procedure

A total of 200 day - old *Arbor acre* breed of broiler chicks were used for this experiment. They were purchased at Yummfy Farms, Ilemona, Kwara state, Nigeria. The chicks were randomly distributed using a Completely Randomised Design (CRD). The experiment lasted for seven weeks. The experimental animals were randomly assigned to five levels of nano Se. Treatment 1 served as the control which had zero (0) level of nano Se. Treatments 2,3,4 and 5 had 0.10, 0.15, 0.20 and 0.25 mg/kg and were tagged $NSe_{0.00,}$ $NSe_{0.10}$, $NSe_{0.15}$, $NSe_{0.20}$ and $NSe_{0.25}$ respectively. Each treatment was replicated thrice with 10 birds per replicate.

3.3 Experimental Diet

The diets used for the experiment both at starter and finisher phases were sourced from Hybrid Feeds Limited, Kaduna, Kaduna state. Preparation of the nano selenium was carried out at the STEP-B, Biotechnology Laboratory, Federal University of Technology, Minna, Niger state. Graded levels of nano Se ranging from 0.10 mg to 0.25 mg per kg

were supplemented as additive into the feed administered to the experimental birds. The chickens were served both feed and water *ad libitum*.

The feed given was subjected to proximate analysis at the Department of Animal Production Laboratory, Federal University of Technology, Minna. The analysis was carried out in line with procedures of the Association of Official Analytical Chemists, A.O.A.C. (2000).

3.4 Housing and Management

The birds were housed in a deep litter system during the period of the study. Prior to their arrival, the pen was washed and disinfected against parasite using Vinkokill at 150 ml per 20 litres of water. Litter material (wood shavings) was evenly spread on the floor to a height of 30 cm. Drinkers and feeders were also washed and disinfected with Vinkokill.

Upon arrival of the chicks, they were weighed in groups and the weight recorded was divided by the number of the chicks in the group to obtain their initial weights and after which they were randomly distributed to the various treatment groups. Anti-stress (vitalyte) was administered to them through drinking water. Each table spoon (20 g) of the vitalyte was diluted into 10 litres of water and then administered. *Gumboro* vaccine was administered twice at 7th and 21st days while *lasota* vaccine was also given at 14^{th} and 28^{th} days. Two hundred doses of these vaccines were diluted in two litres of water in which a sachet of powdered peak milk has been added in order to neutralise the presence of any trace of chlorine and administered after the birds have been starved of water for 12 hours (7pm to 7am). The experiment began when the birds were at three days old.

Table 3.1: Calculated analysis of the experimental diets

Proximate analysis	Starter	Finisher
Crude protein **(%)**	23.00	20.00
Crude fibre **(%)**	4.05	5.53
Ether extract (%)	5.81	5.55
Calcium (%)	1.13	1.11
Available P (%)	0.59	0.58
ME (kcal/kg)	2800.00	3000.00

P = Phosphorus, ME = Metabolizable energy

3.5 Preparation of Nano selenium

Nano Se was produced by biological method of the nano mineral. This is because the biological method of synthesizing nano Se has been proven to be safe, eco-friendly and can be efficiently exploited without the need to conduct a further experiment on the residual effect. It is also believed to be non-toxic, compatible to pharmaceutical and biomedical applications and less time consuming. Scent leave (*Ocimum gratissimum*) extract was used in line with the method described by Jay and Shafkat (2018). This preparation was carried out at the Step B Biotechnological Laboratory, Bosso Campus, FUT Minna.

Freshly harvested scent leaf was gotten along Jamilaville farms (Talba farms), Industrial layout, Minna. 150 g of scent leaf was finely cut and soaked in 600 ml of distilled water at room temperature for 24 hours. The extract was then filtered using Whatman filter paper number 1. 100 ml of 5 mM of sodium selenite was measured into a beaker and placed on a hot plate (model: Jenway 1000). 40 ml of aqueous scent leaf extract was then added into the beaker in a drop wise manner, heated for 30 minutes at 60 ^{0}C until a colour change of sodium selenite was observed. The aqueous solution was then oven dried (model: Drier Box AX – OV73) at 80 ^{0}C until it formed crystals. These crystals were ground to finer particles using pistle and mortar.

The synthesized nano Se was characterised using:

UV-Visible Spectrophotometer:

After 10-15 minutes of color change, this instrument was used to measure the reduction of metallic selenium ions. On a UV-Vis spectrophotometer, a tiny aliquot of the solution

was taken and a wavelength range of 250 nm to 700 nm was measured (Optizon Double beam 3220).

3.6 Data Collection

The methods used and data collected are discussed below:

3.6.1 Growth performance

The different parameters determined for growth performance include the following:

Initial body weight

This is the average live weight of the chick before the commencement of the feeding trial. It was determined thus:

$$\text{Initial body weight} = \frac{weight\ of\ the\ total\ chicks}{total\ number\ of\ the\ chicks}$$

Feed Intake (F.I)

This is the difference between the amount of feed offered to the animal and the feed leftover. A known quantity of feed was supplied to the animal daily and the left over after 24 hours were weighed. This parameter was calculated thus using the formula given by Owen *et al*. (2013):

F.I= Amount of feed offered to the animal – feed left over after 24 hours

Body Weight Gain (BWG)

This is the difference between the final weight and the initial weight. It was calculated thus using the formula given by Owen *et al*. (2013):

BWG= Final weight – Initial weight

Feed Conversion Ratio (FCR)

It is the quantity of feed that will produce one kg weight gain in an animal. It was calculated both weekly and at the end of the experiment using the formula given by Mohapatra *et al*. (2014) below:

$$FCR = \frac{Total\ feed\ intake}{total\ weight\ gain}$$

Mortality

This is the number of birds that died during the period of experiment. The dead birds were recorded for each replicate and their values were expressed as a percentage of the total birds in the replicate. A post mortem analysis to investigate the cause of the mortality was carried out.

3.6.2 Apparent nutrient retention

This is a measure of the relative amount of nutrient present in the gut of an animal from a known quantity of feed consumed. Total collection method was used. This was conducted at both the starter (21 day) and finisher (49 day) phases. Each phase lasted for seven days.

Three chickens were selected randomly from each treatment and allowed to acclimatize for three days to the battery cage that was used. A cellophane paper was placed underneath the battery cage for faeces to drop on it. The selected birds were starved of feed overnight for 12 hours (7:00p.m to 7:00a.m) prior to the collection of the droppings but allowed access to fresh clean water after which they were fed the respective diets for four days. These droppings from each replicate were then scooped, collected daily, oven-dried at 90 ^{0}C and recorded as dry matter. The samples were then analysed for nano Se concentration using AAS atomic absorption spectrometer PinAAcle 900H (Syngistix^{TMb} Inc.) as described by AOAC (2000). The apparent nutrient retention was calculated using the formula as described by Gresakov (2016)

$$\text{Nutrient retention} = \frac{nutrient\ intake\ in\ feed - nutrient\ voided\ in\ faeces}{nutrient\ intake\ in\ feed}\ X\ 100$$

3.6.3 Carcass evaluation

The process of evaluating meat by the producer, meat packers, retailers, and consumers is known as carcass evaluation. It is categorized into two: quality grading and yield grading. This was done at the end of the experimental period that is, 7th week.

Two birds, each having average weights of the treatments were picked randomly from each treatment and starved overnight for 12 hours (7:00pm to 7:00am) but allowed access to fresh clean water. They were weighed individually before slaughtering to obtain their live weight. Parameters like the plucked weight, eviscerated weight, bled weight and the dressing percentage were determined as described by Gresakov (2016). Measurements of the visceral organs and primal parts were carried out. The weight of each primal part and visceral organs were taken and expressed as percentage of the carcass and live weight respectively.

$$\text{Dressing percentage} = \frac{weight\ of\ dressed\ carcass}{live\ weight}\ X\ 100$$

$$\text{Primal cut (\%)} = \frac{weight\ of\ primal\ cut}{carcass\ weight} X\ 100$$

3.6.4 Meat quality characteristics

Meat quality is defined as a set of features or characteristics that determines whether meat is suitable for eating as fresh or stored for a fair amount of time without deterioration (El-Masry *et al*., 2012).

The following parameters were assessed:

pH

This is a measure of the degree of acidity or alkalinity of the meat sample. A pH ranging from 6.0 to 1.0 indicates increasing acidity, 8.0 to 14.0 shows increasing alkalinity while a pH of 7.0 means neutral. Generally, the pH of meat ranges from 5.2 to 7.0. The values

were determined twice using a portable meat pH meter (Hanna H199163) at 0 and 24 hours after slaughtering.

About 10 g of meat was blended with 90 ml of distilled water for two minutes. The pH meter was then inserted in it and the value recorded using the method described by Bowker *et al*. (2014).

Water Holding Capacity

It is the ability of meat to retain its natural and additional moisture during manufacturing, processing, and storage. It is a significant characteristic of fresh meat since it influences both yield and quality of the finished product. It was determined using the filter paper press method.

About 5 g of 24 hours aged meat was homogenized on a metal plate. From this, 300 mg meat was measured and put on Whatman paper number 1, placed between two slides on which a 100 g weight was placed on the top slide for 5 minutes so as to exert downward force and to release water from the meat as described by Abraham and Kumar (2000). The water released by the meat was wetting the paper and the boundary of the wetted area was demarcated using a sharp pencil, measured and reported in percentage of the ratio of the diameter of meat to the diameter of the water wetted paper as described by Mendiratta *et al*. (2008).

$$\text{WHC} = \frac{diameter\ of\ meat}{diameter\ of\ the\ whatman\ paper\ wetted\ area}\ X\ 100$$

Hot Carcass Weight (HCW)

The unchilled weight of the carcass after slaughter is known as the hot carcass weight. This was determined after the chicken's head, intestines, and internal organs have been removed. Both the yield grade and the dressing percentage were determined using this parameter. Three birds per treatment were selected to determine this parameter.

$$\text{HCW} = \frac{Dressing\ percentage}{Live\ weight}\ X\ 100$$

Meat Cooking Yield and Loss

This is the difference between raw and cooked areas of a meat sample reported as a percentage of raw area. Meat samples were taken from the breast area and trimmed to a thickness of 10 mm and a width of 55 mm before and after cooking at 165°C for 10 minutes, with the meat reaching an internal temperature of 70°C. This was carried out according to the procedure stated by Barbera and Sonia (2006).

$$\text{MCS} = \frac{weight\ of\ raw\ meat - weight\ of\ cooked\ area}{weight\ of\ raw\ area}\ X\ 100$$

3.6.5 Sensory Properties of Meat:

This parameter determines the overall acceptability of the meat by consumers. About 100 g of lean meat was taken from the breast of the birds in each replicate. These meat samples were boiled at 80 ^{0}C for 30 minutes in water with 1 g of salt added as crow flies for each treatment and then allowed to cool. A 20-member trained panel of tasters were drawn from the University community and about 15 g of the boiled meat were served to each of them. This evaluation was done according to the method described by Grunert *et al*. (2004).

This characteristic was evaluated using a 9-point hedonic scale ranked thus: 9= like extremely, 8= like very much, 7= like moderately, 6= like slightly, 5= neither like nor dislike, 4= dislike slightly, 3= dislike moderately, 2= dislike very much, 1= dislike extremely.

The meat samples given to the panellists were evaluated for their organoleptic properties such as appearance, juiciness, taste, tenderness, flavour, aroma, texture and general

acceptability. Bottled water was provided for the panellists to rinse their mouth in order to reduce carryover effects.

3.6.6 Gut morphological study

Changes in intestinal processes, such as villi structure and microbial population, have been found to be strongly linked to commercial broiler chickens' efficient feed utilization.

The organs were obtained from the sacrificed birds. This study utilized three birds per treatment, each of which was euthanized by cervical dislocation. The jejunum was taken from the location where the bile duct enters the stomach and Meckel's diverticulum begins.

To remove the digesta, jejunal samples were cut to approximately 3.0 cm lengths and washed with saline solution. The samples were then soaked in 10% neutral-buffering formalin for histology before being dehydrated, cleaned, and embedded in paraffin according to standard histology methods. Six-micron thick sections were cut and mounted on glass slides, then stained with haematoxylin and eosin and viewed under a light microscope.

Other parameters determined under this gut morphological study included the total length of the small intestine, villus height (measured from the tip of the villus to the crypt), crypt depth (measured from the base of the villi to the submucosa) and villus to crypt ratio. This was done as described by Horn *et al*. (2010).

3.6.7 Serum biochemical profile

To enhance serum partition for biochemical analyses, a 5 ml blood sample was taken from the bird's wing vein using a dispensable 5 ml syringe and needle into clean containers (without anticoagulant). The serum was obtained by centrifugation at 3,000

revolutions per minute (rpm) for 10 minutes at a temperature of 28 ^{0}C, then stored at - 20 ^{o}C in a deep cooler until it was required for serum assays.

Following George (2009) biuret response protocol, blood serum was used to quantify Serum Total Protein (STP). Serum proteins react with copper sulphate in sodium hydroxide to form a violet biuret complex, according to the guidelines. Using a DRE 3000 HACH spectrophotometer, the strength of the violet shading was calculated (Hach Inc., USA). As described by George (2009), albumin was measured using a dye-binding technique that depends on the capacity of egg whites to form a stable complex with bromocresol green dye (2009). The absorbance of the samples was measured at 546 nm and 37 °C against a reagent blank. These cylinders and their contents were mixed together and incubated at 37 °C for an hour and a half. Using a DRE 3000 HACH spectrophotometer, the level of egg whites (g/dl) was calculated (Hach Inc., USA). The difference between the total protein concentration and the albumin fraction was used to calculate the serum total globulin concentration. A commercial kit was used to determine creatinine concentration (Creatinine Liquicolor, Germany). Using commercial reagent kits, the components of serum glucose, urea, and cholesterol were measured spectrophotometrically (Thermo Fisher Scientific Inc.).

3.6.8 Immune Response

This is defined as a significant response to an antigen that occurs when lymphocytes identify the antigenic particle as distant and commence the synthesis of antibodies and lymphocytes capable of responding to it and rendering it harmless to the body. Antibody titres against Newcastle Disease were measured in the experimental birds (NCD). Three birds were randomly selected from each treatment and their blood collected at 4th and 8th weeks. The blood was collected from the wing vein of each bird. The serum was isolated by centrifugation at 3000 rpm for 20 minutes and emptied into spotless, clean

plastic vials and stored in deep freeze at -18 ^{0}C to -20 ^{0}C. Serum immune response titres against Newcastle Disease (ND) and Avian Influenza (H9N1) were determined by methods for Hemagglutination Inhibition (HI) test utilizing standard techniques portrayed in World Organization for Animal Health OIE (2009). Antibody titre to infectious bursal disease virus was also determined by commercial ELISA kits (Synbiotics Laboratories, USA), as indicated by producer's guidelines.

3.8 Data Analysis

The data generated from the experiment were subjected to Analysis of Variance (ANOVA) using Statistical Analysis System (SAS, 2015) version 9.3 and where significant differences existed between treatment means, Least Significant Difference (LSD) was adopted to separate the means.

CHAPTER FOUR

4.0 RESULTS

The results of effects of feeding varying dietary levels of nano Se on growth performance of broiler chickens aged 0-4 weeks are presented in Table 4.1a. The results showed that supplementing nano Se in the feed of broiler chickens during the starter phase (0-4 weeks) had effects ($P<0.05$) on all the growth performance parameters measured.

Chickens on dietary $NSe_{0.15,}$ $NSe_{0.20}$ and $NSe_{0.25}$ treatments had similar ($P>0.05$) final body weight and weight gain. Similarly, there were no significant difference ($P>0.05$) in the final and body weight gain of chickens on dietary $NSe_{0.10}$, $NSe_{0.15}$ and $NSe_{0.20}$ treatments. However, chickens on $NSe_{0.25}$ diet had higher ($P<0.05$) final body weight and body weight gain than those on $NSe_{0.00}$ and $NSe_{0.10}$ diets. Also, birds on $NSe_{0.00}$ treatment had lower ($P<0.05$) final body and body weight gain compared to birds on $NSe_{0.10,}$ $NSe_{0.15}$ and $NSe_{0.20}$ treatments.

The feed intake results showed that chickens on dietary treatments $NSe_{0.10,}$ $NSe_{0.15}$ and $NSe_{0.25}$ had similar ($P>0.05$) values. Chickens on $NSe_{0.15}$ and $NSe_{0.20}$ treatments also had similar ($P>0.05$) feed intake values. The feed intake of chickens on $NSe_{0.15}$ were higher ($P<0.05$) than those birds on dietary $NSe_{0.00}$ and $NSe_{0.20}$ treatments.

The results of the feed conversion ratio showed that birds fed diets containing $NSe_{0.15,}$ $NSe_{0.20}$ and $NSe_{0.25}$ had similar ($P>0.05$) values. Similarly, birds on dietary treatments $NSe_{0.10}$ and $NSe_{0.15}$ had similar ($P>0.05$) FCR values. Birds on $NSe_{0.20}$ and $NSe_{0.25}$ treatments had better ($P<0.05$) FCR values compared to those of $NSe_{0.00}$ and $NSe_{0.10}$ treatments.

Birds fed dietary $NSe_{0.10}$ and $NSe_{0.20}$ treatments had similar ($P > 0.05$) mortality values. Birds on $NSe_{0.15}$ and $NSe_{0.25}$ diets also had similar ($P > 0.05$) mortality values. However,

birds fed dietary $NSe_{0.00}$ and $NSe_{0.25}$ treatments had higher ($P < 0.05$) mortality compared to those birds on $NSe_{0.10}$ and $NSe_{0.20}$ treatments.

Results of effects of feeding varying dietary levels of nano Se on the finisher phase (5 – 7 weeks) of broiler birds is presented in Table 4.1b. The results showed that supplementing nano Se in the feed of broiler birds during the finisher phase (5 – 7 weeks) had effects ($P < 0.05$) on all the growth performance parameters measured except the initial weight and mortality.

Birds on dietary treatments $NSe_{0.10}$, $NSe_{0.15}$ $NSe_{0.20}$ and $NSe_{0.25}$ had similar ($P > 0.05$) final body weights and body weight gain. Similarly, there were no significant differences ($P>0.05$) in both the final body weight and body weight gain of birds on dietary $NSe_{0.00}$ and $NSe_{0.10}$ treatments. However, birds on dietary $NSe_{0.15}$, $NSe_{0.20}$ and $NSe_{0.25}$ treatments had higher ($P<0.05$) final body weights and body weight gain than the birds on dietary $NSe_{0.00}$ treatment.

The feed intake of the birds fed diets containing $NSe_{0.00,}$ $NSe_{0.10}$, $NSe_{0.15}$ and $NSe_{0.25}$ treatments had similar ($P > 0.05$) values which were significantly lower ($P < 0.05$) than birds fed $NSe_{0.20}$ treatment. Supplementing nano Se in diets of birds fed $NSe_{0.10,}$ $NSe_{0.15}$ and $NSe_{0.25}$ led to similar ($P > 0.05$) effect on FCR values. Birds fed diets containing $NSe_{0.00,}$ $NSe_{0.10}$, $NSe_{0.15}$ and $NSe_{0.20}$ treatments also had similar ($P > 0.05$) FCR values. However, birds on dietary treatment $NSe_{0.25}$ had better ($P<0.05$) FCR values compared to those on $NSe_{0.00}$ and $NSe_{0.15}$ treatments.

Table 4.1a Growth performance of broiler chickens fed nano selenium supplemented diets aged 0 – 4 weeks

Parameters	$NSe_{0.00}$	$NSe_{0.10}$	$NSe_{0.15}$	$NSe_{0.20}$	$NSe_{0.25}$	SEM	P-value
Initial weight (g)	61.75	61.53	61.27	61.86	62.16	0.227	0.821
FBW (g)	746.48^{c}	961.12^{b}	997.69^{ab}	1052.19^{ab}	1111.50^{a}	0.325	0.000
BWG (g)	684.73^{c}	899.60^{b}	936.42^{ab}	990.32^{ab}	1049.34^{a}	0.325	0.000
Feed intake (g)	1797.47^{c}	2023.82^{a}	1911.78^{ab}	1897.58^{bc}	2015.60^{a}	0.234	0.001
FCR	2.63^{c}	2.25^{b}	2.07^{ab}	1.93^{a}	1.93^{a}	0.071	0.001
Mortality (%)	15.00^{c}	0.00^{a}	2.00^{b}	0.00^{a}	5.00^{bc}	1.552	0.000

^{a,b,c} Means on the same row with different superscripts differ significantly ($P < 0.05$)

FBW= Final Body Weight, BWG= Body Weight Gain, FCR=Feed conversion ratio, NSe = Nano Selenium (mg/kg)

Table 4.1b: Growth performance of broiler chickens fed nano selenium supplemented diets aged 5 – 7 weeks

Parameters	$NSe_{0.00}$	$NSe_{0.10}$	$NSe_{0.15}$	$NSe_{0.20}$	$NSe_{0.25}$	SEM	P-value
Initial weight (g)	749.21	963.19	995.89	1054.19	1200.21	0.094	0.400
FBW (g)	1943.25^{b}	2177.25^{ab}	2244.25^{a}	2233.50^{a}	2325.00^{a}	45.712	0.027
BWG (g)	958.01^{b}	1192.46^{ab}	1259.76^{a}	1249.50^{a}	1339.78^{a}	45.734	0.037
Feed intake (g)	2415.89^{b}	2542.13^{b}	2485.34^{b}	2884.04^{a}	2590.74^{b}	42.984	0.001
FCR	2.60^{b}	2.16^{ab}	1.99^{ab}	2.36^{b}	1.94^{a}	0.085	0.021
Mortality	1.33	0.00	3.00	2.00	1.33	0.593	0.667

^{a,b,c} Means on the same row with different superscripts differ significantly ($P < 0.05$)

FBW= Final Body Weight, BWG= Body Weight Gain, FCR=Feed conversion ratio, NSe = Nano Selenium (mg/kg)

The results of effects of feeding varying dietary levels of nano Se on growth performance of broiler birds at the whole of the experiment (0 – 7 weeks) are presented in Table 4.1c. The results showed that supplementing nano Se in the feed of broiler birds during the experimental trial (0 – 7 weeks) had effects ($P<0.05$) on all the growth parameters measured except the initial weight and FCR

Birds on dietary treatments $NSe_{0.10,}$ $NSe_{0.15,}$ $NSe_{0.20}$ and $NSe_{0.25}$ had similar ($P>0.05$) final body weight and weight gain. Similarly, there were no significant differences ($P>0.05$) in the final body weight and weight gain of birds on dietary treatments $NSe_{0.00}$ and $NSe_{0.15}$. However, birds on treatments $NSe_{0.10}$, $NSe_{0.20}$ and $NSe_{0.25}$ had higher ($P<0.05$) final body weight and body weight gain than the birds on $NSe_{0.00}$ treatment.

The feed intake results showed that birds on dietary treatments $NSe_{0.15}$ and $NSe_{0.25}$ had similar ($P>0.05$) values. Birds on $NSe_{0.10}$ and $NSe_{0.15}$ diets also had similar ($P>0.05$) feed intake values. Similarly, there was no significant difference ($P > 0.05$) in birds fed dietary $NSe_{0.00}$ and $NSe_{0.10}$ treatments. However, birds on $NSe_{0.20}$ treatment had higher ($P<0.05$) F.I values compared to those birds on $NSe_{0.00,}$ $NSe_{0.10,}$ $NSe_{0.15}$ and $NSe_{0.25}$ treatments.

Birds fed dietary $NSe_{0.10}$ and $NSe_{0.25}$ treatments had similar ($P>0.05$) mortality values. Birds fed $NSe_{0.00}$, $NSe_{0.15}$ and $NSe_{0.20}$ treatments also had similar ($P>0.05$) mortality values. However, birds on $NSe_{0.00,}$ $NSe_{0.15}$ and $NSe_{0.20}$ treatments had higher ($P<0.05$) mortality compared to birds on $NSe_{0.10}$ and $NSe_{0.25}$ treatments.

Table 4.1c: Growth performance of broiler chickens fed nano selenium supplemented diets aged 0 – 7 weeks

Parameters	$NSe_{0.00}$	$NSe_{0.10}$	$NSe_{0.15}$	$NSe_{0.20}$	$NSe_{0.25}$	SEM	P-value
Initial weight(g)	61.75	61.53	61.27	61.86	62.16	0.227	0.821
FBW (g)	1943.25^{b}	2177.25^{ab}	2244.25^{a}	2233.50^{a}	2325.00^{a}	45.712	0.047
Weight gain (g)	1881.50^{b}	2115.72^{a}	2182.98^{ab}	2171.64^{a}	2262.84^{a}	45.670	0.047
Feed intake (g)	4213.36^{d}	4382.93^{cd}	4453.90^{bc}	4907.86^{a}	4606.33^{b}	59.503	0.000
FCR	2.24	2.07	2.04	2.26	2.04	0.043	0.148
Mortality (%)	6.25^{b}	0.00^{a}	5.00^{b}	6.25^{b}	1.50^{a}	1.483	0 .000

^{a,b,c,d} Means on the same row with different superscripts differ significantly ($P < 0.05$)

FBW= Final Body Weight, FCR=Feed conversion ratio, NSe = Nano Selenium (mg/kg)

Results of effects of feeding different dietary levels of nano selenium on the apparent nutrient digestibility of broiler birds are presented in Table 4.2. The results showed that feeding supplemental nano Se of varying levels had effects ($P < 0.05$) on only the dry matter (DM) and crude protein (CP) of broiler birds at the starter phase. Supplementing nano Se in the diet of broiler birds had no effects ($P > 0.05$) on other parameters (Ash, ether extract, crude fiber and nitrogen free extract) measured at this phase.

Chickens fed diets containing $NSe_{0.10}$, $NSe_{0.15}$, $NSe_{0.20}$ and $NSe_{0.25}$ treatments had similar ($P > 0.05$) DM values. Values of the DM of chickens fed dietary $NSe_{0.00}$, $NSe_{0.10}$ and $NSe_{0.15}$ treatments were also similar ($P > 0.05$). However, the digestibility values of the DM contents of chickens fed supplemental $NSe_{0.00}$ treatment was significantly lower ($P < 0.05$) than the values recorded for chickens fed supplemental $NSe_{0.20}$ and $NSe_{0.25}$ treatments.

The CP content digestibility of chickens fed supplemental $NSe_{0.00}$, $NSe_{0.10}$ and $NSe_{0.15}$ diets had similar ($P > 0.05$) values. Supplementing $NSe_{0.10}$, $NSe_{0.15}$ and $NSe_{0.20}$ diets to broiler chickens had no significant difference ($P > 0.05$) in CP digestibility. Similarly, there were no effects ($P > 0.05$) in the CP contents digestibility of birds fed $NSe_{0.15}$, $NSe_{0.20}$ and $NSe_{0.25}$ treatments. However, chickens fed dietary $NSe_{0.25}$ treatment had significantly higher ($P < 0.05$) CP digestibility value compared to values recorded for birds fed dietary $NSe_{0.00}$ and $NSe_{0.10}$ treatments.

At the finisher phase, results of the digestibility showed that supplementing nano Se in the diets of broiler birds had effects ($P < 0.05$) on only the DM and Crude fiber (CF) contents whereas supplemental nano Se had no effects ($P > 0.05$) on other parameters (ash, ether extract, crude protein and nitrogen free extract) measured at this phase.

Chickens fed diets containing $NSe_{0.10}$, $NSe_{0.15}$, $NSe_{0.20}$ and $NSe_{0.25}$ had similar ($P > 0.05$) DM digestibility values. The DM digestibility of birds fed dietary $NSe_{0.00}$, $NSe_{0.10}$, $NSe_{0.15}$ and $NSe_{0.20}$ treatments were also similar ($P > 0.05$). However, chickens supplemented with dietary $NSe_{0.25}$ had a DM digestibility value which was significantly higher ($P < 0.05$) compared to those birds on $NSe_{0.00}$ treatment.

The CF digestibility of chickens fed supplemental $NSe_{0.20}$ and $NSe_{0.25}$ diets had similar ($P > 0.05$) values. Similarly, there were no effects ($P > 0.05$) in the CF digestibility in birds fed $NSe_{0.00}$, $NSe_{0.10}$, $NSe_{0.15}$ and $NSe_{0.20}$ diets. However, chickens fed dietary $NSe_{0.25}$ had significantly higher ($P < 0.05$) CF digestible value compared to those birds on $NSe_{0.00}$, $NSe_{0.10}$ and $NSe_{0.15}$ diets.

Table 4.2: Apparent nutrient digestibility of broiler birds fed nano Se supplemented diets

Parameters (%)	$NSe_{0.00}$	$NSe_{0.10}$	$NSe_{0.15}$	$NSe_{0.20}$	$NSe_{0.25}$	SEM	P-value
Starter (0 – 4 weeks)							
Dry matter	79.21^{b}	82.52^{ab}	87.61^{ab}	89.02^{a}	87.20^{a}	1.410	0.024
Ash	76.38	80.05	72.85	75.09	71.79	1.287	0.295
Ether extract	78.94	79.62	79.34	83.78	70.92	1.946	0.357
Crude protein	72.87^{c}	75.28^{bc}	79.74^{abc}	82.79^{ab}	86.22^{a}	1.669	0.039
Crude fibre	78.69	84.51	82.56	79.46	78.92	0.940	0.190
NFE	82.33	82.34	84.12	76.70	75.26	1.797	0.487
Finisher (5–7 weeks)							
Dry matter	81.51^{b}	88.74^{ab}	84.82^{ab}	89.65^{ab}	91.32^{a}	1.418	0.036
Ash	78.18	81.85	74.76	74.89	74.79	1.422	0.470
Ether extract	80.82	81.52	81.25	84.71	83.29	1.791	0.970
Crude protein	81.87	77.38	80.37	84.87	88.32	1.783	0.383
Crude fibre	80.38^{b}	80.23^{b}	80.30^{b}	84.26^{ab}	89.54^{a}	1.171	0.013
NFE	84.56	84.54	86.52	76.87	81.02	1.804	0.520

^{a,b,c} Means on the same row with different superscripts differ significantly ($P < 0.05$)

NFE= Nitrogen free extract. NSe = Nano Selenium (mg/kg)

The results of effect of nano Se supplementation on the carcass weight of *Arbor acre* breed of broiler chickens are presented in Table 4.3. The results showed that feeding different levels of nano Se had effects ($P < 0.05$) on the live weight, carcass weight and dressing percentage of the chickens while other parameters like the slaughter weight and plucked weight were not significantly different ($P > 0.05$).

Birds on dietary treatments $NSe_{0.10}$, $NSe_{0.15}$ $NSe_{0.20}$ and $NSe_{0.25}$ had similar ($P > 0.05$) live weights. Similarly, there were no significant differences ($P>0.05$) in the live weights of birds on dietary $NSe_{0.00}$ and $NSe_{0.10}$ treatments. However, birds on dietary $NSe_{0.15}$, $NSe_{0.20}$ and $NSe_{0.25}$ treatments had higher ($P<0.05$) live weights than the birds on dietary $NSe_{0.00}$ treatment.

The carcass weights and dressing percentage of birds fed diets supplemented with $NSe_{0.10}$, $NSe_{0.15}$ $NSe_{0.20}$ and $NSe_{0.25}$ treatments were similar ($P > 0.05$). There were no significant differences ($P > 0.05$) in both the carcass weights and dressing percentage of birds fed dietary $NSe_{0.00,}$ $NSe_{0.10}$ and $NSe_{0.15}$ treatments. However, birds fed $NSe_{0.20}$ and $NSe_{0.25}$ treatments had higher ($P < 0.05$) values of carcass weights and dressing percentage compared to those broiler chickens on $NSe_{0.00}$ treatment.

The weights of some selected organs as percentage of the live weight showed that feeding broiler diets supplemented with NSe had no effect ($P>0.05$) on all the parameters measured except the lung and gizzard.

Birds fed dietary $NSe_{0.15}$ and $NSe_{0.25}$ treatments had the weights of their lungs to be similar ($P>0.05$); lungs of birds fed supplemental $NSe_{0.10}$ and $NSe_{0.15}$ treatments were also similar ($P>0.05$). The lungs of birds on dietary $NSe_{0.00}$, $NSe_{0.10}$ and $NSe_{0.20}$ treatments also had similar ($P>0.05$) values but these were lower ($P<0.05$) than birds on dietary $NSe_{0.15}$ and $NSe_{0.25}$ treatments. Similarly, the weights of gizzard of birds

supplemented with $NSe_{0.00}$, $NSe_{0.10}$, $NSe_{0.15}$ and $NSe_{0.25}$ dietary treatments were similar ($P>0.05$), their values were higher ($P<0.05$) than the values recorded for birds fed $NSe_{0.20}$ treatment.

Furthermore, the weights of the thighs of birds fed dietary $NSe_{0.10}$, $NSe_{0.15}$, $NSe_{0.20}$ and $NSe_{0.25}$ treatments were similar ($P>0.05$). Similarly, birds fed $NSe_{0.00}$, $NSe_{0.10}$, $NSe_{0.15}$ and $NSe_{0.25}$ also had similar ($P>0.05$) thigh weights. However, birds supplemented with $NSe_{0.20}$ treatment recorded a higher ($P<0.05$) thigh weight compared to those birds fed supplemental $NSe_{0.00}$ treatment.

Table 4.3: Carcass weight of chickens fed nano selenium supplemented diets as percentage of live weight

Parameters (g)	$NSe_{0.00}$	$NSe_{0.10}$	$NSe_{0.15}$	$NSe_{0.20}$	$NSe_{0.25}$	SEM	P-value
Live weight	1941.25[b]	2175.91[ab]	2241.89[a]	2231.99[a]	2324.03[a]	45.712	0.047
Slaughter weight	1734.00	2060.00	1860.00	2135.00	2195.00	79.177	0.351
Pluck weight	1494.00	1985.00	1780.00	1960.00	1950.00	78.062	0.227
Carcass Weight	1114.93[b]	1573.77[ab]	1544.51[ab]	1625.36[a]	1740.78[a]	78.670	0.050
Dressing %	57.43[b]	70.12[ab]	70.93[ab]	72.77[a]	74.87[a]	2.025	0.038
Primal cuts/carcass weight (Percentage of carcass weight, %)							
Breast	13.51	18.53	18.71	18.03	17.23	0.803	0.212
Thigh	8.76 [b]	11.47[ab]	11.50[ab]	11.61[a]	11.00[ab]	0.431	0.049
Wing	12.26	15.32	15.72	14.80	17.63	0.779	0.325
Drumstick	7.03	7.97	8.43	9.32	8.82	0.412	0.561
Organ's weight/live weight (percentage of live weight, %)							
Liver	2.09	1.48	1.42	1.40	1.80	0.129	0.428
Heart	0.41	0.40	0.44	0.52	0.44	0.022	0.529
Lung	0.52[c]	0.60[bc]	0.70[ab]	0.50[c]	0.77[a]	0.037	0.025
Gizzard	2.02 [a]	2.04[a]	2.14 [a]	1.35 [b]	2.10[a]	0.103	0.015
Proventriculus	0.42	0.43	0.45	0.49	0.42	0.023	0.480
Small intestine	2.25	2.09	2.17	2.44	2.41	0.065	0.437
Large intestine	1.89	1.86	1.85	1.84	1.91	0.043	0.992
Pancreas	0.19	0.19	0.20	0.18	0.17	0.007	0.181

[a,b,c] Means on the same row with different superscripts differ significantly ($P < 0.05$)

NSe = Nano Selenium (mg/kg)

The results of the effects of dietary nano Se supplementation on broiler chicken's meat water holding capacity (WHC) and pH at 0 and 24 hours after slaughter are presented in Table 4.4. The results showed that there exist significant differences ($P<0.05$) in the WHC at 0 and 24 hours after slaughter and meat pH at 24 hours after slaughter. However, treatments had no effect ($P > 0.05$) on meat pH at 0 hours after slaughter.

Birds fed diets containing $NSe_{0.00,}$ $NSe_{0.10}$, $NSe_{0.15}$ and $NSe_{0.20}$ had similar ($P > 0.05$) WHC which were significantly lower ($P < 0.05$) than values recorded for birds on $NSe_{0.25}$ diet. At 24 hours after slaughter, meat from birds fed diets containing $NSe_{0.10,}$ $NSe_{0.15}$ and $NSe_{0.25}$ treatments had similar ($P > 0.05$) WHC values. The values recorded for meat from birds fed dietary $NSe_{0.10}$, $NSe_{0.15}$ and $NSe_{0.20}$ treatments were also similar ($P > 0.05$). Similarly, there exists no difference ($P > 0.05$) in the WHC of meat from birds fed diets containing $NSe_{0.00}$ and $NSe_{0.20}$ treatments. However, meat from birds on $NSe_{0.00}$ had lower ($P < 0.05$) WHC compared to those on $NSe_{0.10}$, $NSe_{0.15}$ and $NSe_{0.25}$ treatments.

The pH of meats from birds obtained at 24 hours after slaughter was influenced ($P<0.05$) by dietary treatments. The meat from birds fed diets containing $NSe_{0.00}$, $NSe_{0.10}$, $NSe_{0.20}$ and $NSe_{0.25}$ treatments had similar ($P>0.05$) pH values. The meats from birds supplemented with diets containing $NSe_{0.00,}$ $NSe_{0.10}$ and $NSe_{0.15}$ treatments also had similar ($P>0.05$) pH values. However, meat from birds on $NSe_{0.20}$ and $NSe_{0.25}$ diets had higher ($P < 0.05$) pH values than those on $NSe_{0.15}$ diet.

Table 4.4: Water Holding Capacity and pH of meat from broiler chickens fed nano Se supplemented diet

Parameters	$NSe_{0.00}$	$NSe_{0.10}$	$NSe_{0.15}$	$NSe_{0.20}$	$NSe_{0.25}$	SEM	P-value
Water holding capacity							
0 hour after slaughter	0.42^{b}	0.94^{b}	1.24^{b}	0.52^{b}	4.02^{a}	0.374	0.001
24 hours after slaughter	0.23^{c}	1.60^{ab}	2.11^{ab}	0.89^{bc}	2.41^{a}	0.255	0.012
pH							
At 0 hours	5.96	5.70	5.50	6.63	6.30	0.173	0.144
At 24 hours	5.73^{ab}	5.73^{ab}	5.50^{b}	6.13^{a}	6.03^{a}	0.083	0.021

^{a,b,c} Means on the same row with different superscripts differ significantly ($P < 0.05$)

NSe = Nano Selenium (mg/kg)

The results of effects of feeding varying nano Se supplementation on both the meat cooking yield and cooking loss of broiler chicken's meat are presented in Table 4.5. The results showed that supplementing different levels of nano selenium had significant effects ($P<0.05$) in all parameters measured except those of the raw samples.

Birds on dietary treatments $NSe_{0.10,}$ $NSe_{0.15}$, $NSe_{0.20}$ and $NSe_{0.25}$ had similar ($P>0.05$) meat cooking yield values but their values were higher ($P < 0.05$) than meat from birds fed dietary $NSe_{0.00}$ treatment. Meat from birds fed diet containing $NSe_{0.10,}$ $NSe_{0.15}$ and $NSe_{0.25}$ treatments had similar ($P>0.05$) meat cooking loss values, their values were significantly lower ($P<0.05$) from the meat of birds fed dietary $NSe_{0.00}$ treatment. Meat from birds fed dietary $NSe_{0.20}$ was significantly lower ($P < 0.05$) compared to the meat of birds on $NSe_{0.00}$ and $NSe_{0.10}$ supplemented diets.

Table 4.5: Cooking yield and loss from meat of broiler birds fed nano Se supplemented diets

Parameters	$NSe_{0.00}$	$NSe_{0.10}$	$NSe_{0.15}$	$NSe_{0.20}$	$NSe_{0.25}$	SEM	P-value
Raw (g)	30.00	30.21	29.70	29.70	29.66	0.109	0.478
Yield (%)	93.95^{b}	96.27^{a}	95.68^{a}	97.86^{a}	95.78^{a}	0.371	0.001
Loss (%)	6.05^{c}	3.73^{b}	4.32^{b}	2.14^{a}	4.22^{b}	0.509	0.001

^{a,b,c} Means on the same row with different superscripts differ significantly ($P < 0.05$)

NSe = Nano Selenium (mg/kg)

The results of effects of feeding different levels of Nano selenium (NSe) on the sensory properties of meat from broiler birds are presented in Table 4.6. The results showed that supplementing NSe in the diets of broiler chickens had significant effects ($P<0.05$) on both meat juiciness and overall acceptability while other parameters (colour, flavour and tenderness) were not affected ($P>0.05$) by NSe supplementation.

Birds on dietary $NSe_{0.10}$, $NSe_{0.15,}$ $NSe_{0.20}$ and $NSe_{0.25}$ treatments had similar ($P>0.05$) meat juiciness values. There were no significant differences ($P>0.05$) in the juiciness of meat from broiler birds on $NSe_{0.00}$, $NSe_{0.20}$ and $NSe_{0.25}$ treatments. However, meat from birds on $NSe_{0.10}$ and $NSe_{0.15}$ diets were juicier ($P < 0.05$) than meat from birds fed dietary $NSe_{0.00}$ treatment. The overall acceptability results showed that meat from broiler birds on $NSe_{0.10}$, $NSe_{0.15,}$ $NSe_{0.20}$ and $NSe_{0.25}$ diets had similar ($P > 0.05$) overall acceptability values. Similarly, meat from birds on $NSe_{0.00,}$ $NSe_{0.10,}$ $NSe_{0.15}$ and $NSe_{0.25}$ diets also had similar ($P > 0.05$) overall acceptability values. However, meat from birds on $NSe_{0.20}$ diets had higher ($P < 0.05$) overall acceptability values than those on $NSe_{0.00}$ diet.

Table 4.6: Sensory evaluation of the meat of broiler chickens fed supplemental nano Selenium diets

Parameters (%)	$NSe_{0.00}$	$NSe_{0.10}$	$NSe_{0.15}$	$NSe_{0.20}$	$NSe_{0.25}$	SEM	P-value
Colour	2.50	1.50	1.50	1.50	1.50	0.213	0.574
Flavour	2.50	2.00	2.50	1.50	2.00	0.277	0.855
Juiciness	1.00^{b}	2.50^{a}	2.50^{a}	1.50^{ab}	2.00^{ab}	0.233	0.042
Tenderness	2.00	2.00	1.50	1.00	2.50	0.249	0.454
Overall Acceptability	1.00^{b}	1.50^{ab}	2.00^{ab}	2.50^{a}	1.50^{ab}	0.213	0.029

^{a,b} Means on the same row with different superscripts differ significantly ($P < 0.05$)

NSe = Nano Selenium (mg/kg)

Results of effects of feeding different levels of nano Selenium on the haematological parameters of broiler birds are presented in Table 4.7. The results showed that feeding different levels of nano Se to broiler birds had effects ($P < 0.05$) on the haemoglobin, Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC), neutrophils and lymphocytes while monocytes, eosinophils, basophils, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were not influenced upon nano selenium supplementation.

Chickens on dietary $NSe_{0.10,}$ $NSe_{0.15}$ and $NSe_{0.20}$ had similar ($P > 0.05$) haemoglobin, PCV, RBC and lymphocytes values. Similarly, there were no significant difference ($P > 0.05$) in the values of haemoglobin, PCV and lymphocytes for chickens fed dietary $NSe_{0.00,}$ $NSe_{0.10,}$ $NSe_{0.15}$ and $NSe_{0.20}$ treatments. However, birds fed dietary $NSe_{0.00}$ and $NSe_{0.25}$ treatments had lower ($P < 0.05$) haemoglobin, PCV, RBC and lymphocytes than those birds on $NSe_{0.20}$ diet.

Chickens fed dietary $NSe_{0.10}$ and $NSe_{0.20}$ treatments had similar ($P > 0.05$) white blood cells (WBC) values. Chickens on $NSe_{0.00}$, $NSe_{0.10}$, $NSe_{0.15}$ and $NSe_{0.25}$ treatments also had similar WBC values. However, the values of the WBC in broiler birds on $NSe_{0.20}$ diet were higher ($P < 0.05$) than the values recorded for birds supplemented with dietary $NSe_{0.00,}$ $NSe_{0.15}$ and $NSe_{0.25}$ treatments. Furthermore, the neutrophils of birds fed supplemental $NSe_{0.00}$, $NSe_{0.10}$, $NSe_{0.15}$ and $NSe_{0.20}$ were not significantly different ($P > 0.05$). Birds on $NSe_{0.00}$, $NSe_{0.10}$, $NSe_{0.15}$ and $NSe_{0.25}$ diets also had similar ($P > 0.05$) neutrophils values. However, chickens fed dietary $NSe_{0.25}$ treatment had lower ($P<0.05$) neutrophil values compared to those fed dietary $NSe_{0.20}$ treatment.

Table 4.7: Haematological parameters of broiler birds fed nano Se supplemented diets

Parameters	$NSe_{0.00}$	$NSe_{0.10}$	$NSe_{0.15}$	$NSe_{0.20}$	$NSe_{0.25}$	SEM	P-value	Ref. range
Haemoglobin	11.95^{b}	12.80^{ab}	12.30^{ab}	14.50^{a}	11.10^{b}	0.435	0.050	7 – 13 g/dl
PCV	36.00^{b}	38.50^{ab}	37.00^{ab}	44.00^{a}	33.50^{b}	1.348	0.050	30 – 40 %
RBC	5.95^{b}	6.40^{ab}	6.15^{ab}	7.30^{a}	5.55^{b}	0.224	0.049	3.4–4.6 x 10^{3} µl
WBC	4.05^{b}	4.50^{ab}	4.10^{b}	5.35^{a}	3.65^{b}	0.217	0.047	12 – 30 x 10^{3} µl
Neutrophils	27.50^{ab}	29.50^{ab}	29.00^{ab}	31.50^{a}	26.00^{b}	0.746	0.039	30 – 70 %
Lymphocytes	55.00^{b}	61.00^{ab}	59.00^{ab}	64.00^{a}	55.00^{b}	1.364	0.033	28 – 72 %
Monocytes	2.50	2.50	3.00	3.00	2.00	0.163	0.275	2 – 5 %
Eosinophils	1.00	1.00	1.00	1.00	1.00	0.000	0.061	0 – 1 %
Basophils	1.00	1.00	1.00	0.50	1.00	0.100	0.486	0 – 2 %
MCV	60.45	60.15	60.25	62.85	60.00	0.554	0.542	90–140 fL
MCH	20.05	20.00	20.00	20.07	20.00	0.139	0.500	33–47 pg/cell
MCHC	33.15	33.20	33.10	32.95	33.30	0.000	0.668	26-35 g/dl

^{a,b} Means on the same row with different superscripts differ significantly ($P < 0.05$)

NSe = Nano Selenium (mg/kg), PCV= Packed Cell Volume, RBC= Red Blood Cell, WBC= White Blood Cell, MCV= Mean Corposcular Volume, MCH= Mean Concentration Hemoglobin, MCHC= Mean Corposcular Haemoglobin Concentration. Source of reference range: Schalm *et al*., (1975), Bounous and Stedman (2000)

The results of effects of feeding NSe on the serum biochemical profile of broiler birds are presented in Table 4.8. The results showed that supplementing NSe in the diets of broiler birds had effects ($P<0.05$) on both urea and creatinine while glucose, cholesterol and total protein were not influenced on nano Se supplementation.

The results showed that the urea contents in birds fed dietary $NSe_{0.00}$, $NSe_{0.10}$ and $NSe_{0.15}$ treatments had similar ($P>0.05$) values. Birds on $NSe_{0.00}$ and $NSe_{0.20}$ diets also had similar ($P > 0.05$) urea values. The urea contents of birds fed supplemental $NSe_{0.20}$ and $NSe_{0.25}$ diets were also similar ($P > 0.05$). However, birds on $NSe_{0.25}$ diet had the lowest ($P<0.05$) urea contents compared to broiler birds on $NSe_{0.00}$, $NSe_{0.10}$ and $NSe_{0.15}$ diets.

Supplementing $NSe_{0.00,}$ $NSe_{0.10,}$ $NSe_{0.15}$ and $NSe_{0.20}$ treatments in the diets of broiler birds resulted in similar ($P>0.05$) creatinine values. Similarly, there were no differences ($P > 0.05$) in the creatinine values of broiler birds fed supplemental $NSe_{0.00}$, $NSe_{0.10}$ and $NSe_{0.25}$ diets. Birds on $NSe_{0.25}$ diet, however, had lower creatinine values than those birds on $NSe_{0.15}$ and $NSe_{0.20}$ diets. The albumin values of birds fed dietary $NSe_{0.00}$, $NSe_{0.15}$, $NSe_{0.20}$ and $NSe_{0.25}$ treatments were similar ($P>0.05$) while those fed dietary $NSe_{0.00}$, $NSe_{0.10}$ and $NSe_{0.15}$ treatments were also similar ($P>0.05$). However, birds fed supplemental $NSe_{0.10}$ diet had lower ($P < 0.05$) albumin values than those on $NSe_{0.20}$ and $NSe_{0.25}$ diets.

Table 4.8: Effects of feeding nano Selenium on some selected serum biochemical indices of broiler birds

Parameters	$NSe_{0.00}$	$NSe_{0.10}$	$NSe_{0.15}$	$NSe_{0.20}$	$NSe_{0.25}$	SEM	P-value	Ref. range
Glucose	4.97	5.13	5.53	5.00	4.80	0.286	0.963	2.8–8.9 mmol/dl
Urea	6.30^{ab}	7.30^{a}	7.50^{a}	5.50^{bc}	4.37^{c}	0.358	0.004	1.9-12.5 mg/dl
Creatinine	6.33^{ab}	7.00^{ab}	8.33^{a}	7.33^{a}	4.00^{b}	0.524	0.046	0.1 – 14.0 u/l
Cholesterol	0.667	0.60	0.77	0.60	0.70	0.032	0.462	1 – 4 mg/m
Total Protein	6.70	6.63	6.20	6.83	6.33	0.097	0.205	3.0 – 4.9 mg/dl
Albumin	2.70^{ab}	2.33^{b}	2.97^{ab}	3.10^{a}	3.30^{a}	0.116	0.042	1.17–2.74 g/dl

^{a,b,c} Means on the same row with different superscripts differ significantly ($P < 0.05$)

NSe = Nano Selenium (mg/kg)

Source of reference range: Anadon (2006)

Table 4.9 shows the effect of feeding different levels of nano Se on the immunological parameters of broiler chickens. The results showed that supplementing nano Se in the feeds of broiler birds had effects ($P<0.05$) on spleen, immunoglobulin G (Ig G) and immunoglobulin A (Ig A) measured. However, the thymus, bursa and immunoglobulin M were not influenced ($P>0.05$).

Birds fed dietary $NSe_{0.00}$, $NSe_{0.10}$ and $NSe_{0.20}$ treatments had similar ($P>0.05$) spleen values. Birds on $NSe_{0.00,}$ $NSe_{0.10,}$ $NSe_{0.15}$ and $NSe_{0.25}$ diets also had similar ($P>0.05$) spleen values. Birds fed supplemental $NSe_{0.20}$ diet, however, had higher ($P < 0.05$) spleen values compared to those birds on $NSe_{0.15}$ and $NSe_{0.25}$ treatments.

The results of immunoglobulin G (Ig G) showed that birds fed diets containing $NSe_{0.00}$, $NSe_{0.10}$, $NSe_{0.15}$ and $NSe_{0.25}$ treatments had similar ($P>0.05$) values. There were no significant differences ($P>0.05$) in the lg G of broiler birds fed dietary $NSe_{0.00}$, $NSe_{0.10}$, $NSe_{0.15}$ and $NS_{0.20}$ treatments. However, birds fed supplemental $NSe_{0.25}$ treatment had higher ($P<0.05$) numbers of Ig G than those fed $NSe_{0.20}$ treatment.

Birds in treatments $NSe_{0.00}$, $NSe_{0.10}$, $NSe_{0.15}$ and $NSe_{0.25}$ had similar ($P>0.05$) values of the lg A. The birds on $NSe_{0.20}$ and $NSe_{0.25}$ diets also had similar ($P > 0.05$) Ig A values. However, birds fed dietary $NSe_{0.20}$ treatment had lower ($P<0.05$) Ig A values than birds fed dietary $NSe_{0.00}$, $NSe_{0.10}$ and $NSe_{0.15}$ treatments.

Table 4.9: Response of some immunological parameters on broiler chickens fed nano Se supplemented diets

Parameters (%)	$NSe_{0.00}$	$NSe_{0.10}$	$NSe_{0.15}$	$NSe_{0.20}$	$NSe_{0.25}$	SEM	P-value
Thymus	2.95	2.72	2.62	3.21	2.41	0.165	0.654
Bursa	2.06	1.93	2.08	2.06	1.77	0.062	0.503
Spleen	1.91^{ab}	1.92^{ab}	1.56^{bc}	1.99^{a}	1.49^{b}	0.072	0.049
IgG	4.00^{ab}	3.98^{ab}	3.94^{ab}	3.62^{b}	4.43^{a}	0.231	0.048
IgA	2.62^{a}	2.85^{a}	2.74^{a}	1.82^{b}	2.55^{ab}	0.133	0.050
IgM	1.93	2.25	2.11	1.74	2.11	0.080	0.319

^{a,b} Means on the same row with different superscripts differ significantly ($P < 0.05$)

NSe = Nano Selenium (mg/kg), IgG= Immunoglobulin G, IgA= Immunoglobulin A, IgM =Immunoglobulin M.

Presented in Table 4.10 are the results of effect of feeding different levels of nano Se on the gut morphology of broiler chickens. The results showed that supplementing nano Se in the feeds of broiler birds had effects ($P<0.05$) on only the crypt depth while other parameters (villus height, epithelium thickness, villus width and villus to crypt ratio) showed no significant differences ($P > 0.05$) upon supplementation of dietary nano Se in the feeds of broiler chickens.

Birds fed dietary $NSe_{0.00}$, $NSe_{0.10}$, $NSe_{0.20}$ and $NSe_{0.25}$ mg/kg treatments had similar ($P > 0.05$) crypt depth values. Chickens administered dietary $NSe_{0.00}$, $NSe_{0.10}$, $NSe_{0.15}$ and $NSe_{0.20}$ mg/kg treatments also had the values of their crypt depth to be similar ($P > 0.05$). However, birds fed dietary $NSe_{0.25}$ mg/kg treatment had significantly higher ($P < 0.05$) crypt depth values compared to birds fed $NSe_{0.15}$ mg/kg dietary treatment.

4.10: Gut morphological parameters of broiler birds fed dietary nano Se supplemented diets

Parameters	$NSe_{0.00}$	$NSe_{0.10}$	$NSe_{0.15}$	$NSe_{0.20}$	$NSe_{0.25}$	SEM	P-value
Villus height	263.10	251.64	250.08	241.92	245.19	3.550	0.427
Crypt depth	64.29^{ab}	67.05^{ab}	55.55^{b}	65.59^{ab}	74.45^{a}	2.144	0.050
Epithelium thickness	5.71	5.90	5.42	6.19	5.54	0.130	0.392
Villus width	28.91	32.94	29.11	28.33	29.60	0.714	0.278
Villus to crypt ratio	5.12	5.06	5.60	5.83	4.76	0.215	0.576

[a,b] Means on the same row with different superscripts differ significantly ($P < 0.05$)

NSe = Nano Selenium (mg/kg)

CHAPTER FIVE
5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Nano Selenium dietary supplementation at 0.25 mg/kg significantly improved both final body weight (FBW) and body weight gain (BWG) at the starter phase of the experiment. As the level of nano Se increases, it was seen that these parameters also increased. These findings could be due to higher requirements of broilers to selenium as reported by Ahmadi *et al*. (2018) who worked on varying levels of supplemental nano Se using 180 1-day old male Ross 308 chicks at the rate of 0.00 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg and 0.5 mg/kg and discovered that supplementation of nano Se in the diets of Ross 308 broiler chicks improved their body weight gain. The result of the present study is similar with that of Dlouha *et al*. (2008) who reported improvement in body weight with selenium dietary supplementation at the level of 0.3 mg/kg for broiler chickens. Similarly, Hu *et al*. (2012) found that body weight gain was improved linearly as the level of nano Se increased in the diet from 0.15 to 1.20 mg/kg. On the other hand, Cai *et al*. (2012) indicated no significant differences in weight gain of broilers fed diets supplemented with 0.2 to 2.0 mg nano Se per kg of diet. The reason for the differences in the present work and those authors having contrary results could be due to higher doses of nano Se supplementation used by these authors. It might be an indication that the optimal level requirement of nano Se was lower than what was used by the authors.

The feed intake (F.I.) of broiler birds was significantly improved upon the supplementation of varying levels of dietary nano Se. This could be due to the differences that exist in the assimilation of the element in tissues of poultry which is largely dependent on the dietary Se source (Surai *et al*., 2006). The result of this study is in agreement with those of Yang *et al*. (2012) and Bagheri *et al*. (2015) who reported differences in the F.I. of birds fed supplemental selenium diets. On the contrary, similar

studies carried out by Prasoon *et al*. (2018) who worked on effects of dietary supplementation of organic, inorganic and nano Se on antioxidant status of Giriraja chicken showed no significant differences in the F.I. of broiler birds fed nano Se supplemental diet. The reason for the variance could be attributed to the differences in breeds of broiler birds used for the study.

Dietary nano Se supplemented at 0.20 and 0.25 mg/kg of diet at the starter phase enhanced feed conversion ratio (FCR) when compared to the control. This could be attributed to the impact of the body weight and feed intake since FCR is a ratio of body weight gain and feed intake. This result is in agreement with that of Ahmadi *et al*. (2018) who reported an improvement in FCR when chickens were fed supplemental dietary nano Se at the starter phase. Furthermore, Zhou and Wang (2011) observed that final body weight and FCR were significantly improved in the groups supplemented with nano Se as compared with the control in birds reared under thermoneutral and high ambient temperature conditions. These results are, however, contrary to those reported by Downs *et al*. (2000), Peric *et al*. (2009), Rao *et al*. (2013) and El-Deep *et al*. (2016) who all recorded no significant differences in body weight and FCR of chickens fed nano Se supplemented diets at the starter phase of their experiments. The differences may be due to the higher dosage of nano Se fed or the conditions of rearing as seen in El-Deep *et al*. (2016) who fed 0.30 mg/kg nano elemental selenium and 0.30 mg/kg of sodium selenite to birds raised in high ambient temperature ($35 \pm 1\ ^{0}C$).

Supplementing dietary nano Se in the feed of broiler birds significantly affected the mortality rates of the birds as it had an irregular pattern across the treatments. However, higher number of mortality was recorded for birds fed the basal diet compared to those birds supplemented with nano Se. This could be attributed to the higher immunity in

broilers fed nano Se supplemented diets as selenium is known to increase the immune level of broiler birds.

Dietary nano Se supplementation significantly improved both the FBW and the BWG of broiler chickens at the growing phase of the experiment as compared to the control. These could be because broiler birds require more selenium in their feed as reported by Ahmadi *et al*. (2018). The results of the present study are in accordance with those of Zhou and Wang (2011) who recorded an improved final BW, daily BW gain, FCR and survival rate, when nano-Se was supplemented at 0.10, 0.30 and 0.50 ppm in the diets of Guangxi Yellow chicken compared to the control group. Researches carried out by Sevcikova *et al*. (2006), Wang and Xu (2008), Wang (2009) and Ahmadi *et al.* (2018) also shown an improvement in FBW and BWG of chickens supplemented with dietary nano-Se at the grower phase. On the contrary Cai *et al*. (2012) indicated no significant difference in weight gain, feed intake and FCR in broilers fed diet supplemented with 0.3 to 2.0 mg/kg nano-Se. Similar results had also been shown by Mei-Sheng (2005), Payne and Southern (2005), Songbai *et al*. (2015) and Prasoon *et al* (2018) where they could not record an improvement in growth parameters of broiler chickens at the grower stage. The variance in the results compared to the current study may be attributed to the high dietary levels of Se in control diet which masked the effect of supplemental Se (Zhou and Wang, 2011).

Varying levels of nano Se in the diet of broiler chickens significantly influenced the feed intake (F.I.) of broiler chickens during the grower phase of the experiment. This could mean that nano Se has a way of stimulating feed intake, hence, rendering the feed more palatable. The result of this study is in line with those of El-Deep *et al.* (2016) who observed that 0.3 mg/kg nano Se improved F.I. when birds were fed 0.0, 0.3 mg/kg of nano Se and 0.3 mg/kg of Sodium Selenite (SSe) under thermoneutral and high ambient temperature conditions. On the contrary, studies conducted by Tayeb and Quader (2012),

Cai *et al.* (2012), Prasoon *et al.* (2018) and Niu *et al*. (2009) recorded no significant differences in the F.I. of broiler chickens during the grower phase of their experiments. The reason for the differences may have to do with the breed and environment in which the authors carried out their studies.

The addition of nano Se at the levels of 0.10, 0.15 and 0.25 mg/kg in the diets of broiler chickens improved the FCR during the grower (5-7 weeks) phase of the experiment. This could be attributed to high body weight observed in birds fed nano selenium supplemented diets. The results of the present study are in line with those of Salim *et al*. (2015) who fed five sources of selenium namely sodium selenite (NaSe), selenomethionine (Se-yeast), zinc-L-selenomethionine (Zn-Se-Meth), powder form of nano Se and liquid form of nano Se at the levels of 0.15 and 0.30 ppm to broiler chickens and observed significant difference at the grower phase. Similar trend was also observed in the studies conducted by El-Deep *et al*. (2016), when dietary nano-Se was supplemented at 0.3 mg/kg in the diets of broiler chickens under thermoneutral and high ambient temperature conditions. Safdari-Rostamabad *et al*. (2017) who supplemented dietary nano-Se at 1.2 mg/kg observed that nano-Se supplementation alleviated the adverse effects of heat shock on the FCR of heat-stressed broilers. Contrarily, Prasoon *et al*. (2018) reported no significant difference in birds fed (50, 150 and 300 ppb) nano-Se diet. Studies on FCR in broilers as influenced by nano-Se supplementation at 0.0, 0.3, 0.5, 1.0, or 2.0 mg/kg to the basal diet by Cai *et al*. (2012) revealed no significant differences. Moghaddam *et al*. (2017) and Li *et al*. (2018) also reported no significant difference in the FCR of chickens fed 0.3 mg/kg as organic and nano Se diets respectively at the growing phase. The reason for the differences between the results obtained from the current study and those authors reported could be attributed to the temperature of the environment in which the study was carried out as it had been established that

environmental temperature is a key parameter used in determining the FCR since it is a factor of feed intake.

Supplementing dietary nano-Se significantly improved both the final body weight and the body weight gain of the broiler chickens from 0 – 7 weeks of the experiment. This is a similar trend from that obtained during 0 – 4, 5 – 7 and 0 – 7 weeks. This might implies that 0.25 mg/kg of nano Se is adequate for all phases of broiler chickens growth. This is similar to the findings of Salim *et al*. (2015) who recorded significant differences in both the FBW and BWG of broiler chickens at 0 – 7 weeks of the experiment. Similarly, studies by Ibrahim *et al*. (2019) showed a significant increase in body weight and body weight gain of Ross broiler chicks given a supplemental selenomethionine (Se-Met) and nano-Se diets when compared with birds in groups fed selenite selenium (SeS). They discovered that when Se-Met or nano-Se was added to diets, there was increase body weight and gain in proportion to the increase in dietary Se levels.

Supplementation of nano-Se in the diet of broiler chicks showed a significant difference in the F.I. across the treatment groups of birds aged 0 – 7 weeks. This could be due to the numerous roles selenium play in growth (Yoon *et al*., 2007; Wang and Xu 2008) of broiler birds. This is similar to the results obtained by Yang *et al*. (2012), Ravindran and Elliot, (2017) and Zia *et al*. (2017) who reported higher F.I. in dietary Se supplemented groups of broiler chickens. On the other hand, studies carried out by Cai *et al*. (2012), Liu *et al*. (2015), Prasoon *et al*. (2018), Ahmadi *et al*. (2018) and Ibrahim *et al*. (2019), showed no significant differences in the F.I of broiler chickens aged 0 – 8 weeks supplemented with dietary nano Se. This may have to do with the environment in which the authors carried out their studies as it has been established that at low temperature, the birds eat more while at high temperature, the birds eat less.

Supplementing nano Se in the diets of broiler birds significantly affected their mortality rate. Birds fed the basal diet recorded higher mortality rate than those birds in the nano Se supplemented group. This could be due to the differences in the form of Se in the diet as higher absorption and assimilation rates which subsequently increases the level of the immune system of birds has been reported in nano Se diets compared to either organic or inorganic forms.

There have been numerous studies on the relationship between selenium and growth performance (El-Deep *et al*., 2016; Ahmadi *et al*., 2018), carcass components (Naik *et al*., 2015; Konieczka *et al*., 2015), and haematological parameters (Konkov *et al*., 2015), but there is a paucity of data on the effect of nano Se dietary supplementation on the apparent nutrient digestibility.

In the present study, the varying levels of dietary nano Se had influence on the apparent nutrient digestibility of broiler birds both at the starter and finisher phases of the experiment.

At the starter phase, birds fed supplemental nano Se diet had higher dry matter (DM) digestibility implying that birds were able to digest the DM more compared to the birds fed basal diet. Furthermore, the birds fed $NSe_{0.15}$, $NSe_{0.20}$ and $NSe_{0.25}$ diet had higher crude protein digestibility value when compared to other dietary treatments. This may be due to the numerous roles played by selenium in the growth of poultry birds as reported by Yoon *et al*. (2007).

Broiler birds fed nano Se supplemented diets had higher DM digestibility than the birds fed basal diet during the finisher phase. Similar trend was observed for crude fibre (CF) as birds on nano Se supplemented diets digested the CF more than those in the control group. This could be due to monogastric species' high intestinal selenium absorption,

which is highly dependent on the form of selenium (Youcef *et al*., 2013). Attest to this result is the crypt depth observed in this study which revealed that nano Se supplemented diet enhanced the crypt depth. Furthermore, nano particles have been reported to have a larger surface area and thus, strong adsorbing ability than both organic and inorganic forms (Wang *et al*., 2007; Zhang *et al*., 2008). This may be a reason why birds in groups fed nano Se supplemented diets had higher apparent nutrient digestibility than those birds in the control group.

In the present study, the varying levels of nano Se had significantly affected the relative weights of some organs and cut off parts of the meat of broiler chickens. The group fed 0.15 mg/kg nano Se dietary treatment exhibited higher relative weights in the organs and cuts off parts when compared to other treatment groups indicating that this level enhanced carcass yield and dressing percentage. Mineral buildup in tissues serves as an indicator for mineral utilization, according to Liao *et al*. (2012). Due to the obvious importance of these minerals in human diets, nutritionists have been working to increase Se levels in human foods by modifying dietary Se sources and livestock Se levels (Wang and Xu, 2008). It's also been proved that Se-rich meat is juicier, crispier, and more attractive (Suchy *et al*., 2014). When Se was administered in nano form against sodium selenite, Wang *et al*. (2012) observed a greater transport and uptake of Se by broiler intestinal cells (SeS). Se in human food has been set at a safe level of 0.4 mg/day (Adeniyi and Agoreyo, 2018). With their recommendation, the level used in this study is safe because the level used is below 0.4 mg/day.

Dressing percentage is a function of how much meat a carcass will yield as it is based on the relationship between the dressed carcass weight and live animal weight after removing both the skin and internal organs. The results of the present study showed that groups supplemented with nano Se diet had improved carcass yield and dressing

percentage compared to those birds fed the basal diet. This could be attributed to the higher transport, uptake and better utilisation of nano form of Se by the broiler intestinal cells when compared to other forms of Se as reported by Wang *et al*. (2012). Similar trend was observed by Salim *et al*. (2015) who reported improved dressing percentage in the meat of broiler birds fed liquid nano Se compared to those birds fed sodium selenite, Zinc-selenomethionine and selenium yeast diets.

Supplementing nano Se in the diets of broiler chickens led to a significant increase in the relative weights of their thighs. This could be because the weight of thigh is a function of both the carcass yield and dressing percentage which were improved in birds on nano Se supplemented diets. Similar results have been reported by Sevcikova *et al*. (2006), Ahmadi *et al*. (2018) who all recorded significant improvement in the relative weights of the thighs of chickens fed dietary supplemental nano Se.

Supplementing dietary nano Se at 0.25 mg/kg resulted in improved relative weights of both lungs and gizzard of broiler meats. Though there is limited information on the gizzard and lungs of birds as influenced by nano Se supplementation. However, the bigger gizzard weight seen in birds fed supplemented nano Se diet might be the reason the birds on nano Se diets had higher feed intake. This is contrary to the reports of Ahmadi *et al*. (2018) who found no significant differences in the relative weights of gizzard and lungs as affected by the supplementation of different levels of dietary nano Se. The differences could be attributed to the differences in the breeds of chickens used for the study as Ahmadi *et al*. (2018) used Ross 308 chicks while *Arbor acre* chicks was used for the present study.

The ability of a meat to maintain its inherent and added moisture during manufacture, processing, and storage is known as its Water Holding Capacity (WHC). If the WHC in

raw poultry meat is low, consumers may notice a reduction in aesthetic appeal and palatability, as well as a reduction in protein functionality, ingredient retention, and product yield for processors (Bowker and Zhuang, 2016).

The present study demonstrated increased WHC as the level of dietary nano Se increased to 0.25 mg/kg. This could be due to the metabolic conversion of glucose to lactic acid in postmortem muscle being delayed with nano Se supplementation, therefore enhancing the WHC of meat as reported by Oliveira *et al*. (2014). Lambert *et al*. (2001) found that lactic acid accumulation in the muscles, combined with a cessation of blood circulation that causes cellular hypoxia, results in a decrease in pH after slaughter, as well as changes in cell membrane permeability and a decrease in WHC.

Other studies showed that WHC is affected by organic Se (Peric *et al*., 2009) and nano Se (Zhou and Wang, 2011). Lisiak *et al*. (2014) reported higher WHC in pork when pigs were fed organic Se supplemented diets. On the contrary, results reported by Soliman *et al*. (2020) showed no significant differences in the WHC of hubbard broiler birds supplemented with different concentrations of nano Se. Furthermore, studies by Mohammad *et al*. (2019) showed no significant differences in the WHC of the meat of broiler chickens fed different forms of Se supplemented diets. The variance of these results and that obtained from the current study could be due to differences in the form of feeding or broiler birds used.

The pH of a meat is a measure of the degree of acidity or alkalinity of the meat sample. A pH ranging from 6.0 to 1.0 indicates increasing acidity, 8.0 to 14.0 shows increasing alkalinity while a pH of 7.0 means neutral.

The results of the present study shows that dietary nano Se significantly improved the pH of the meat of the broiler chickens at 24 hours post mortem while its supplementation had

no effect on the pH at 0 hour post mortem. Though at both periods, that is 0 and 24 hours post mortem, the pH values obtained were still in the normal range of 5.2 to 7.0. This is in agreement with the findings of Li *et al*. (2017), Ibrahim *et al*. (2019) and Soliman *et al*. (2020) who all recorded significant improvements in the pH of meat from broiler birds fed supplemental dietary nano Se. On the contrary, studies carried out by Mohammad *et al*. (2019) recorded no significant differences in the pH of broiler meat fed dietary Se of various forms while studying the comparative effects of dietary organic, inorganic and nano Se complexes and rosemary essential oil on performance, meat quality and selenium deposition in muscles of broiler chickens. The reason for the variance could be as a result of the inclusion or effect of the rosemary essential oil.

The supplementation of nano Se in the diets of broiler birds resulted in significant differences in the carcass yield and cooking loss of meat gotten from broiler chickens. Birds in treatments supplemented dietary nano Se had higher carcass yield values which were significant compared to the values gotten from the meat of birds fed basal diet. This could be that the rate at which nano Se is transported in the broiler intestinal cells is higher than the basal diet as reported by Wang *et al*. (2012). The result of this study is in variance with those reported by Bakhshalinejad *et al*. (2018) who recorded no significant differences in the carcass yield of birds fed supplemental dietary selenium. This might be because the authors used organic Se in their diets.

Furthermore, results of the current study shows that birds fed nano Se supplemented diets had lower cooking loss compared to their counterparts fed the basal diet. This could be explained by its higher bioavailability as nano particles had been reported to possess larger surface area and strong adsorbing ability (Zhang *et al*., 2008; Wang *et al*., 2017). Similar results have been reported by Yang *et al*. (2012), Li *et al*. (2017) and Zaki and Hassan, (2019) who observed that supplementing nano Se in the diets of broiler chickens

led to decreased meat loss compared to those of control treatment. The findings of Miezeliene *et al*. (2011), on the other hand, showed that Se supplementation in broiler chicken diets had no effect on the percentage of cooking loss of breast and thigh chicken meat. Boiago *et al*. (2014) also reported that the levels of Se supplementation had no effect on the cooking loss of broiler chicken meat. This could be due to the differences in the breeds of broiler birds used for the study.

The sensory evaluation results measures the overall acceptability of a product by consumers. The results of the current study show that supplementing dietary nano Se in the feed of broiler birds influenced both the juiciness and overall acceptability of their meat. This could be because Se rich meat has been proven to be juicier and better looking (Suchy *et al*., 2014). This result is in agreement with that reported by Khan *et al*. (2017) who observed significant improvement in the sensory characteristics of breast meat gotten from broiler birds fed Se supplemented diets compared with those recorded from the control. However, the result of this study is at variance with the findings of Zaki *et al*. (2019) who recorded no significant differences in both meat juiciness and overall acceptability of meat gotten from broiler birds fed supplemental Se diets. Similar studies by Haug *et al*. (2007) and Miezeliene *et al*. (2011) did not record significant differences on the sensory attributes of cooked meat gotten from broiler chickens fed Se supplemented diets. The reason for the variance that exists between the results of the present study and the findings of these authors having contrary results could be attributed to the differences in the form of Se used.

Adejumo (2004) asserted that haematological indices are still a good indicator of an animal's physiological health, and that this has a favorable relationship with the animal's nutritional status.

Supplementation of nano Se at 0.20 mg/kg feed led to higher haemoglobin, PCV, RBC, WBC and lymphocytes when compared with the control group. Above the level, that is, at 0.25 mg/kg, there was a decrease in the values of these parameters. This might be an indication that 0.20 mg/kg of nano Se supplementation was adequate for these parameters. This implies that at higher doses of nano selenium inclusion, there might be a reduction in the concentration of haemoglobin, PCV, RBC and lymphocytes in the blood of broiler birds. These results are in agreement with the findings of Biswas *et al*. (2011), Fawzy *et al*. (2016) and Dalia *et al*. (2017) who all reported significant differences in the haemoglobin concentration of broiler birds supplemented with nano Se diet. On the contrary, studies carried out by Chen *et al*. (2014), Boostani *et al*. (2015) and Okunlola *et al*. (2015) showed no significant differences in the haemoglobin concentration of broiler birds upon supplementation of nano Se in their diets. The reason for the variance could be as a result of variations in the genetic make-up of the animals used for the study.

Haemoglobin, PCV, monocyte, basophil and MCHC results obtained in this study are within the normal ranges as referenced from the studies conducted by Schalm *et al*. (1975) and Bounous and Stedman (2000) except for birds fed dietary nano selenium 0.25 mg/kg treatment. The results will vary due to a several factors, including the fact that most typical referenced values were developed in temperate countries, whose data may not accurately reflect tropical animal characteristics due to variances in environmental conditions and genetic diversity (Onunkwo, 2018).

Birds fed diets supplemented with nano Se at 0.10, 0.15 and those fed the basal diet produced a significantly higher quantity of urea compared to those birds fed supplemented nano selenium 0.20 and 0.25 mg/kg diet. This might implies that the effect of nano Se at lower levels of 0.10 and 0.15 mg/kg diet has similar impact to those of the

conventional forms of Se in *Arbor acre* broiler birds. This is similar to the results of Hassan *et al*. (2020) who reported a significant difference in the amount of urea produced by broiler chickens upon supplementation of nano Se while studying selenium and nano selenium ameliorations in two breeds of broiler chickens exposed to heat stress. Supplementing nano selenium in the feed of broiler birds also influenced their creatinine values. Supplementation of nano Se in the diet of broiler chickens produced a quadratic pattern across the treatment levels. However, birds fed 0.25 mg/kg dietary nano Se had lower creatinine values but not significant with birds fed the control and 0.10 mg/kg diet. Higher creatinine values are an indication that the kidney are not functioning well. Attest to this result is the better growth performance recorded for birds fed 0.25 mg/kg nano Se supplemented diets. This is similar to the findings of Hassan *et al*. (2020) who observed significant differences in the values of creatinine of broiler birds fed nano selenium supplemental diet. However, this is in contrast to the results of Salim *et al*. (2015) who reported that increasing the supplemental Se level from 0.3 to 0.45 ppm in broiler diets could not cause any significant difference in plasma creatinine level. Similarly, Ibrahim *et al*. (2019) recorded no significant differences in the creatinine values of broiler birds fed 0.3, 0.45 and 0.6 mg/kg of nano Se while studying the effect of dietary modulation of Se form and level on performance, tissue retention and quality of frozen stored meat and gene expression of antioxidant status in Ross broiler chickens. The reason for the differences with the current study could either be as a result of administration of high dosage of nano Se or the usage of different breeds of broiler chickens by Ibrahim *et al*. (2019).

The results of the current study showed that the albumin levels in the blood followed an irregular pattern upon nano selenium supplementation. The pattern was however, significantly different across the treatment means. Experimental birds fed 0.25 mg/kg

nano Se diet were observed to have a higher value of albumin level, though statistically similar to those observed in birds fed the basal, 0.15 and 0.20 mg/kg diet. Attest to this is the higher F.I and BWG recorded in birds fed 0.25 mg/kg nano Se supplemented diet. This is in agreement with the results of Ahmadi *et al*. (2018), Hassan *et al*. (2020) who recorded significant differences in the levels of albumin in the blood of broiler chickens fed nano Se supplemented diets. The serum parameters reported were within the normal range as referenced in the works of Anadon (2006).

Se is necessary for immunological development, according to studies, and its deficiency in broiler chickens could result in a weak immune response (Pal, 2017). It is a structural component of at least 25 selenoproteins that regulates a variety of biological functions such as oxidative stress, redox, and other crucial cellular processes in nearly all tissues and cell types throughout the body, including those involved in innate and adaptive immune responses (Dalia *et al*., 2017, Dalgaard *et al*., 2018). The bone marrow, thymus, liver, lymph nodes, and spleen are among the key immune organs that exhibit the presence of Se, according to Huang *et al*. (2012).

In the present study, the supplementation of dietary nano Se in the feed of broiler chickens significantly improved spleen, immunoglobulin G, Ig G and immunoglobulin A, Ig A. This could be attributed to the enhanced activity of cytokines as a result of nano Se supplementation. Nano minerals have a larger surface area, larger active surface centers, more catalytic efficiency, transfer capability, and higher surface absorption and stability than other forms of selenium, resulting in a better immunological response (Payne and Southern, 2005). When these cytokines are released, nutrient absorption and cell development improve, and immunogenic chemicals are produced (Grivennikov *et al*., 2010). This is in agreement with Zhang *et al*. (2012) who reported that the supplementation of Se in the chicken diets improved their immunological parameters.

However, these results are in contrary to those of Rao *et al*. (2013) who reported that supplementing various concentrations (0, 100, 200, 300 or 400 µg/kg diet) of organic Se to broiler chickens had no influence in the production of antibodies that are specific for Newcastle disease virus vaccine. The differences might be because the authors were interested mainly in Newcastle disease.

Furthermore, the supplementation of dietary nano Se at 0.20 mg/kg diet led to improved relative weight of the spleen of broiler birds. This could be due to the fast speed of nanomaterial transport and uptake compared to other forms seen in the basal diet. This improves the performance of lymphoid organs by increasing the activity of glutathione peroxidase (Sadeghain *et al*., 2012). This finding is in agreement with those of El-Said and Tag-El-Din (2017) and Shabani *et al*. (2019) who all recorded a significant improvement in the spleen of broiler chickens fed dietary nano Se. On the contrary, Swain and Johry (2000) and Cai *et al*. (2012) revealed no significant differences in the relative weight of spleen in broiler chickens fed varying levels of dietary nano Se. This may be due to the higher supplementation of nano Se made by these authors.

Supplementing dietary nano Se in the diet of broiler birds at the rate of 0.20 mg/kg diet led to a significant lower Ig A of broiler birds compared to birds fed the basal diet. Ig A is the first line of defence in the resistance against infection. This might implies that the birds when not diseased, thus, there was no need for its production. Selenium is well known to have influence in the immune response of broiler chickens and when administered in nano form, there is increased absorption and transport. This is in agreement with the result obtained by Dalia *et al*. (2017) who also reported a significant difference in the IgA while studying the influence of bacterial organic selenium on blood parameters, immune response, selenium retention and intestinal morphology of broiler chickens. However, in the works of Cai *et al*. (2012), feeding nano selenium

supplemented diets to broiler chickens produced no significant effect on the immunoglobulin A. This may be due to the higher dosage of nano Se supplementation in the feed administered to the broiler chickens.

The study of the gut of broiler birds, which includes the intestine and caecum, is known as gut morphology. Changes in intestinal functions such as villi structure and microbial population have been linked to the efficient utilization of feed by commercial broiler chickens, according to studies. The ability to express selenoproteins is found in about a quarter of the gut microbiome, and selenium availability in microbiological conditions influences their expression (Kasaikina *et al*., 2011).

In the present study, supplementation of dietary nano Se had effect on only the crypt depth of broiler birds. Other gut morphological parameters studied produced no significant effect upon supplementation of dietary nano Se in the feeds of broiler chickens. Results of the study showed that birds supplemented with dietary nano Se at 0.25 mg/kg had higher values of crypt depth. This might be an indication that the birds on this treatment had higher capacity to accomodate ingesta since the deeper the crypt depth, the more feed it will absorb. This is similar to the results of Jessica *et al*. (2009) who reported a significant difference in the crypt depth while studying the effect of dietary Se on small intestine villus integrity in Reovirus-challenged broilers. Similar trend was observed by Hamzekolaei *et al*. (2018) who also reported a significant difference in the crypt depth while studying the effect of nano Se and organic Se in comparison with vitamin C on growth performance, small intestine morphology and lipogenesis in broiler chickens.

5.2 Conclusions

From the results of this study, the following conclusions could be made:

The supplementation of nano Se in the diet of *Arbor acre* broiler birds at the level of 0.25 mg/kg significantly influenced the Final Body Weight, Body Weight Gain, Feed Intake and Feed Conversion Ratio during the starter phase (0 – 4 weeks). Similarly, the Final Body Weight, Body Weight Gain and Feed Conversion Ratio were also influenced by the supplementation of the test ingredient during the grower phase (5 – 7 weeks). Furthermore, supplementing dietary nano Se at 0.25 mg/kg influenced the Final Body Weight, Body Weight Gain and Feed Intake at 0 – 7 weeks compared to the control.

Birds fed dietary nano Se at the level of 0.25 mg/kg had better ($P<0.05$) carcass weight, dressing percentage, WHC, meat yield, cooking loss, haemoglobin and PCV when compared to the control.

Results of the serum biochemical profile shows that the value of the urea in birds fed 0.25 mg/kg nano Se supplemented diet was significantly different compared to those fed the basal diet.

Birds fed 0.20 mg/kg nano Se supplemented diets had better immune response compared to those birds fed the basal diet.

Birds fed 0.25 mg/kg nano Se supplemented diet had deeper crypt depth compared to birds in the control group.

5.3 Recommendations

The following are recommendations of the study:

Broiler birds can be fed with nano Se supplemented diet at the level of 0.25 mg/kg to boost their growth performance and carcass quality.

In order to boost the immune system of broiler birds, nano Se should be supplemented at 0.20 mg/kg diet.

5.4 Contribution to knowledge

The research gives insight on the effects of varying dietary levels of nano selenium on growth performance, gut morphology, haematology, immunity and carcass parameters of broiler chickens. The findings made from the study showed that feeding broiler chickens diets supplemented with nano selenium at 0.25 mg/kg significantly improved body weight gain (22.62.84 g) compared to the control (1881.50 g). Supplementation of nano selenium at 0.25 mg/kg diet also resulted in better dressing percentage (74.87 %) compared to the control (57.42 %). Broiler chickens fed 0.20 mg/kg nano selenium supplemented diet had better dry matter digestibility values (89.65 %) as against the control (81.51 %).

The information from this study may be useful in formulating and preparing the feeds for broiler chickens as nano selenium has been proven to possess high surface activity and strong absorbing ability as against the conventional forms of selenium which is either organic or inorganic forms.

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