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# Evaluation of haematology, serum biochemistry and immune competence of broiler chickens supplemented with dietary zinc and selenium nanoparticles

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**ABSTRACT:** The study evaluated the effect of dietary supplementation of nano zinc and selenium on haematology, serum biochemical parameters and immune function of broiler chickens. Seven hundred and sixty-eight (768) one-day-old Arbor acre broiler chicks were arranged in a 4 × 4 factorial layout in a Completely Randomized Design based on dietary zinc and selenium nanoparticles into sixteen treatments consisting of four replicates with 12 birds per replicate for an experimental duration of 29 days. Birds were reared on deep litter and fed basal diets enriched with four levels of nano zinc (20, 30, 40 and 50 mg/kg NZn) and four levels of nano selenium (0.10, 0.15, 0.20 and 0.25 mg/kg NSe). On day 49, blood samples were collected from two randomly selected birds in each replicate to determine haematology, serum biochemical profile and immunoglobulin concentrations. Data generated were subjected to a two-way analysis of variance using the General Linear Model of Statistical Package for Social Science (SPSS version 16.0). Results indicated haemoglobin, PCV, RBC, neutrophils, lymphocytes and glucose, urea creatinine, cholesterol, albumin, nitrogen, aspartate amino transaminase and alkaline phosphatase were significantly ($p<0.05$) improved by 50 mg of Zn/kg with 0.15 mg of Se/kg and their values are within the range of reference values. Birds fed up to 50 mg/kg NZn revealed enhanced thymus, bursa of Fabricius, spleen and IgM. Similarly, 0.10 to 0.25 mg/kg NSe improved the bursa of Fabricius, IgA, IgG and IgM. Dietary supplementation of nano zinc and selenium improved blood biochemical parameters and increased immunity without any detrimental effect on the birds' health.

**Keywords:** Broiler chickens, haematology, immune competence, nano zinc, selenium, serum biochemical profile.

## INTRODUCTION

The pursuit of safe and high-quality animal protein has led scientists to enrich poultry products (meat and egg) with essential bioactive minerals such as Zinc (Zn) and Selenium (Se) nanoparticles via the fortification of poultry feed. This strategy promotes poultry health, growth, survival and optimal productive efficiency ultimately benefiting the overall economy of production (Salim *et al*., 2012). Zinc and selenium are essential microelements in poultry nutrition for maximum performance (Haiam *et al*., 2020). Zinc (Zn) is required for the normal functioning of the animal body, it plays a crucial role as a cofactor of more than 300 enzymes, structural and regulatory functions in antioxidant, nucleic acid synthesis, cell proliferation, protein synthesis, protein and carbohydrate metabolism, and enzymatic activities in the living system (Fayiz *et al*., 2021). It is essential for growth, skeletal development and immune competence (Bartlett *et al*., 2003). Zinc deficiency in poultry can lead to a reduction in weight gain, skeletal malformations, poor bone mineralisation and immunological dysfunctions (Burrell *et al*., 2004). Therefore, supplementing poultry diets with zinc and selenium has been shown to have a positive effect on broiler chickens,

with tissue uptake increasing linearly with dietary levels (Bartlett *et al*., 2003). Zinc is essential for the normal functioning of the immune system by increasing the counts of thymocytes and peripheral T cells which serve as natural killer cells (Dardenne and Bach, 1993). It also boosts the production of neutrophils, and antibodies and improves the functions of macrophages (Kidd *et al*., 2000).

Despite their importance, minerals remain one of the most deficient minerals in broiler chickens, with a significant reduction in productivity Chrastinova *et al*., 2016). In living organisms, a natural antioxidant system protects cells from the harmful effects of reactive oxygen species (Surai, 2006). This antioxidant system comprises natural fat-soluble vitamin E, ascorbic acid, and antioxidant enzymes: such as glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) which play crucial roles in protecting cells from reactive oxygen species (ROS) by reducing free radicals and preventing the peroxidation of lipids (Surai *et al*., 2016). Selenium (Se) a micro-mineral is considered a functional part of the antioxidant system through selenoproteins. About 25 selenoproteins have been identified as containing a selenocysteine amino acid as its unique structural part (Huang *et al*., 2017). Consequently, selenocysteine is essential for the activity of selenoenzymes including glutathionzze peroxidases, thioredoxin reductases, and iodothyronine deiodinases (Chen *et al*., 2003). Glutathione peroxidase is a selenium-dependent enzyme involved in the antioxidant system which helps to control free radical formation reducing hydrogen peroxide and lipid peroxide (Rotruck *et al*., 2009). In addition, selenoprotein plays an antioxidant function in chicken myoblasts and its synthesis is affected by the nutritional level of the Se (Yao *et al*., 2013). selenium supplementation is necessary to overcome various stressors (Sahoo *et al*., 2014a). However, Selenium bioavailability depends on many factors such as intestinal absorption and biological activation and physical form (Surai, 2006). According to Chen *et al*. (2003), a selenium deficient diet can decrease the expression of 25 selenoproteins in the muscular proventriculus of broilers chickens. While the National Research Council (1994) recommended 30 mg/kg zinc and 0.15 mg/kg selenium for broiler chickens as reported by Burrell *et al*. (2004) and Liu *et al*. (2011). this might not be adequate to support maximum performance as reported by Du *et al*. (2007).

Recently, the emergence of nanotechnology has enabled the development of nano zinc and selenium as feed supplements to improve the efficiency of trace minerals in broiler chickens (Geetha *et al*., 2020). The nano-sized particles have higher potential than the conventional ones due to their high surface area to volume ratio which enables nanoparticles to be effective in very small amounts and absorbable more quickly than inorganic and organic minerals (Sawosz *et al*., 2009).

There is widespread concern within the animal industry regarding the minimum recommended levels of zinc and selenium (NRC, 1994) as they may not be sufficient for optimal productivity. However, excessive dietary inclusion of these minerals can be toxic to the animals as well as their products. Conversely, suboptimal levels may result in low productivity efficiency and selenium deficiency syndrome (Hayes, 2008; Cai *et al*., 2012). The bioavailability and utilization of zinc and selenium are significantly influenced by their physical form. Common zinc and selenium sources in broiler chicken feed include inorganic and organic forms such as salts, selenium-yeast, and seleno-methionine (Bartlett *et al*., 2003). Therefore, efforts are geared at exploring alternative mineral sources that meet optimal requirements while ensuring safety for consumption. Limited information in the literature exists on comparing inorganic, organic or a combination of nano zinc and selenium supplementation in broiler chicken diets. Therefore, the study aimed to investigate the effect of dietary nano zinc and selenium supplementation on haematology, serum biochemical profile and immune competence of Arbor acre broiler chickens.

## MATERIALS AND METHODS

### Description of the study location

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm of the Department of Animal Production, School of Agriculture and Agricultural Technology, Federal University of Technology, (FUT) Minna, Niger State, Nigeria. The town is situated between latitude 9$^{o}$28’ and 9$^{o}$37’ North, and longitude 6° 23’ and 6°33’ East. It has an annual rainfall of 1000 – 1500 mm and average temperature of 32°C. It is located in the Southern Guinea Savannah Vegetation Zone of Nigeria (Minna Meteorological Station, 2022). In addition, blood and immune response analyses were carried out at Step ‘B’ Drug and Vaccine Discovery Laboratory, Bosso Campus of the same institution.

### Source of experimental materials

The feed ingredients for experimental feed formulation were procured from Hybrid Feed Limited, Kaduna State, Nigeria. The Africa scent leaves used for nano synthesis were sourced from Olericulture garden of the FUT, Minna. All other reagents and equipment used were obtained from Step ‘B’ Drug and Vaccine Discovery Laboratory, Bosso Campus, Federal University of Technology, Minna.

### Preparation of the plant extract

Fresh leaf of African scent (*Ocimum gratissimum)* leave were harvested from the Olericulture garden, FUT, Minna, between June and August, 2020. About 50 g of scent leaf were thoroughly washed and rinsed with distilled water and then chopped into small pieces the chopped leave were mixed with 500 ml distilled water in 1000 ml beaker

and heated at 100°C for 30 minutes using a heater and magnetic stirrer (Model: Jenway 1000). after cooling to room temperature, the mixture was filtered using Whatman paper no. 1 and then centrifuged at 4000 rpm for 10 minutes (Life Assistance Scientific UK) to remove the plant residues and impurities as outlined by Marye and Inbathamizh (2012). The final plant extract was stored in the refrigerator for further uses.

**Green synthesis of zinc and selenium nanoparticles**

The zinc and selenium nano particles were synthesized using a biosynthetic method which is efficient, eco-friendly and non-toxic (Marye and Inbathamizh, 2012). This biological method also ensures safe and rapid product recovery without residual effects. The synthesis of nano zinc and selenium were carried out in line with the protocols described by Jay and Shafkat (2018). For the synthesis of zinc oxide nanoparticles, 100 ml of scent leaf aqueous extract with initial pH of 4.6 was mixed with 2 g of sodium hydroxide (NaoH) and diluted with distilled water to buffer the pH to 13.00 using (Hanna pH meter). Thereafter, 50 ml of 0.2 mM solution of zinc nitrate was slowly added at drop wise into 400 ml of scent leave extract in the beaker until the solution colour changed to yellowish white. The mixture was heated and stirred vigorously with a magnetic bar for 30 minutes at room temperature using heater-stirrer (Hot Plat, model: Jenway 1000). Liquor of 2 ml was taken from zinc nanoparticle into a small plastic bottle to measure the wavelength from 200 nm to 800 nm using UV-visible Spectrophometer (UV.1800, Shimadzu, Japan). It was centrifuged at 4000 rpm for 10 minutes at room temperature using Centrifuge 80-2 (Life assistance scientific UK). The obtained product was calcined at 100°C in an oven for 8 hours.

Synthesis of selenium nano particle, 100 ml of 0.4 mM concentration of sodium selenite was gently added to 40 ml of scent leaf aqueous extract and stirred slowly with a spatula until the solution colour change from light grey to dark brown. The beaker containing the solution mixture and the magnetic bar was thoroughly heated and stirred for 30 minutes at room temperature with Hot Plat ((model: Jenway 1000). Liquor of 2 ml was drawn from selenium nanoparticle solution into a small plastic bottle to measure the wavelength from 200 to 800nm using UV-visible Spectrophometer (UV.1800, Shimadzu, Japan). The product obtained was calcined at 100°C in an oven for 36 hours for crystal formation. Nano zinc and selenium obtained were subjected to zetasizer analysis to determine particle size using Microtec Zetatrac (NPA 152, Korea).

**Experimental diets and design**

The experimental diets were formulated to meet the nutritional requirements of broiler chickens in line with the recommendation of National Research Council (NRC, 1994). Proximate analyses of the diets were carried out using the method of Association of Official Analytical Chemists (2000). The ingredients and composition of the experi-mental diets is presented in Table 1.

A total of seven hundred and sixty-eight (768) day old broiler chicks of Arbo Acre plus, were procured from Yammfy Farm hatchery at Ilemona, Kwara state. The chicks were randomly assigned to 16 treatment groups, arranged in a 4 × 4 factorial design, with four levels of zinc (Zn) and four levels of selenium (Se) in a completely randomized design. Each treatment group was replicated four times, with 12 birds per replicate. The treatments designation is presented in Table 2.

**Management of the birds**

Before the arrival of the broiler chicks, the pen, drinkers, feeders, and brooders were cleaned, washed, disinfected with Izal® and fumigated using formaldehyde and potassium permanganate. The birds were reared on deep litter. The building was demarcated into 64 compartments with wood, net and ceiling board. Fresh wood shavings were spread to a depth of 5 cm and other appliances such as pilot lights, drinkers, feeders and heating devices (Abacha charcoal stove) were provided. All the groups were provided with similar environmental, and management conditions throughout the experimental period of 49 days. On arrival, the broiler chicks were weighed individually, sorted, and randomly allocated to 16 treatments with 4 replicates of 5 birds in each group for 49 days. Immediately after the chicks were allotted to their respective replicates, they were given an anti-stressor (Vitalyte®) via drinking water. Adequate feeding and drinking space of 0.02 $m^2$ was provided to all the birds throughout the experimental period. The birds were given free access to fresh, clean drinking water throughout the experimental period. A weighed amount of experimental feeds was offered every morning and evening to each replicate and the leftover feeds were collected and weighed separately at a daily interval. From this data, the average daily feed consumption per bird in each replicate was calculated. The immunization against Infectious Bursa of Fabricius l Disease (IBD Intermediate strain) and Newcastle Disease was observed. Birds were vaccinated using live vaccines Gumboro on the $7^{th}$ and Lasota 14th days. It was followed by booster doses of Gumboro on the 21st day and Lasota 28th day through drinking water.

**Data collection**

***Haematological indices***

Blood samples of 4 ml were collected from randomly selected chickens via cardiac exposure and right

**Table 1.** Ingredients composition of the experimental diets.

| Ingredients % | Starter phase | Finisher phase |
|---|---|---|
| Maize | 47.00 | 55.00 |
| Soybean cake | 34.00 | 26.00 |
| Fish meal | 3.00 | 3.00 |
| Wheat offal | 11.00 | 11.00 |
| Palm oil | 1.00 | 1.00 |
| Limestone powder | 1.00 | 1.00 |
| Bone meal | 2.00 | 2.00 |
| Salt | 0.25 | 0.25 |
| Lysine | 0.25 | 0.25 |
| Methionine | 0.25 | 0.25 |
| **Vitamin/mineral premix | 0.25 | 0.25 |
| Total weight (kg) | 100.00 | 100.00 |
| Calculated analysis | | |
| Crude protein (%) | 23.00 | 20.00 |
| Crude fibre (%) | 4.05 | 5.53 |
| Ether extract (%) | 5.81 | 5.55 |
| Ca (%) | 1.13 | 1.11 |
| Avail P (%) | 0.59 | 0.58 |
| ME (kcal/kg) | 2800.00 | 3000.00 |

Premix supplied per Kg of diet: Vit. A, 2.5iu; Vit D3, 0.5iu; Vit E, 0.0057mg; Vit. K, 0.0005mg; Vit, B1, 0.00045mg; Vit B2, 0,0013mg; pantothenic acid, 0.0018mg; Vit. B12, 0.000005mg; Folic acid, 0.00018mg; Biotin, 0.000015mg; Choline chloride, 0.075mg; Cobalt, 0.00005mg; Copper, 0.00075mg; Iodine, 0.00025mg; Iron, 0.0025mg; Manganese, 0.01mg; Selenium, 0.00005mg; Zinc, 0.0075mg; Antioxidant, 0.00031mg.

ventricular apex puncture using 5 ml disposable syringe and hypodermic needle. The blood samples were put into sterilized vial bottles containing Ethylene Diaminetetra-acetic Acid (EDTA) for the haematological study. The following blood parameters determined includes Packed Cell Volume (PCV), Red Blood Cells (RBC), White Blood Cells (WBC), Haemoglobin (Hb) concentration, Haematocrit (HCT), Mean Corpuscular Volume, (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC). These parameters were analyzed according to the standard procedures described by Mohammed *et al*. (2018).

## Serum biochemical indices

A second blood sample of (4 ml) was collected from the same birds using disposable 5 ml syringe and hypodermic needle into plain bottles (without anticoagulant) to enhance serum separation for biochemical studies. Serum was obtained by centrifugation at 3,000 revolutions per minute (rpm) for 10 minutes at ambient temperature of 28°C and store at 4°C in LG deep freezer until required for the determination of biochemical parameters. The blood serum was used to determine Serum Total Protein (STP) following the biuret reaction procedure of George (2009). The principle of this reaction is that serum proteins react with copper sulphate in sodium hydroxide to form a violet biuret complex. The intensity of the violet colour was measured using a DRE 3000 HACH spectrophotometer (Hach Inc., USA). Albumin was determined using a dye-binding technique that utilizes the ability of albumin to form a stable complex with bromocresol green dye as described by George (2009). The absorbances of the samples were measured against reagent blank at 546 nm in temperature of 37°C. These tubes and their contents were mixed and incubated for 90 minutes at 37 C. Estimation of albumin level (g/dl) was obtained using a DRE 3000 HACH spectrophotometer (Hach Inc., USA). Creatinine concentration was determined using a commercial kit (Creatinine Liquicolor, Germany). Serum glucose, nitrogen urea and cholesterol constituents were determined by spectrophotometrically (Thermo Fisher Scientific Inc.,) using commercial reagent kits. Serum hepatic enzymes, namely Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) were determined using commercial kits as described by Yang *et al*. (2012). The analysis was carried out at the Biochemistry Laboratory of the Niger State Ministry of Livestock and Fishery Development, Veterinary Clinic, Bosso, Minna.

## Immune response

Experimental broiler chickens were assessed for immune organs and serum immunoglobulins against Newcastle Disease (ND). Two birds from each replicate were randomly

selected for blood collection at 7th week of age. 4 ml of blood samples were collected from each bird using a syringe and a hypodermic needle from cardiac exposure and right ventricular puncture. After clotting, serum was separated by centrifugation at 3000 rpm for 20 minutes and decanted into clean, sterile plastic vials and stored in a Thermacool deep freezer at 4°C. The birds were euthanized and the thymus, bursa of fabricius and spleen were removed and weighed with electron digital weighing scale 275 (Ace Inc., Jaipur, India) to determine immune organ indexes using the formula described by Song *et al*. (2021):

$$\text{Immune organs index (g/kg)} = \frac{\text{Weight of immune organs (g)}}{\text{Live body weight (kg)}}$$

This was determined at Step 'B' Drug and Vaccine Discovery Laboratory, Bosso Campus, Federal University of Technology, Minna, Nigeria. Serum samples were analyzed by enzyme-Linked Immunosorbent Assay using flock check index (IDEXX) ELISA test kit. The assay was conducted following the guidelines provided by the manufacturer: 1:500 into a test tube. One hundred microlitre samples of diluted serum were dispensed in duplicate to appropriate wells of Newcastle disease antigen coat microlitre plates. It was incubated for 30 minutes at room temperature. The solution contents of wells were aspirated and washed six times with 350 µl distilled water. Thereafter, 100 µl of goat antichicken peroxidase conjugated second was added for 30 minutes at room temperature. The wells were rewashed six times and 100 µl of 3,3,5,5 Tetramethyl benzidine solutions were introduced. It was further incubated for 15 minutes at room temperature. The reaction was stopped with the addition of 100 µl of 2M $H_2SO_4$ to each well. The optical density of the plates was read by an automatic DR 200B microplate plate reader at 360 nm (Hiweli Diatek Instrument Co., China). This was done according to the standard methods described in World Organization for Animal Health (OIE, 2009), at the National Veterinary Research Institute, Vom, Jos, Plateau State, Nigeria.

### Ethical approval

This research was conducted in strict accordance with the recommendations and guidelines of Federal University of Technology, Minna ethical committee for the care and handling of laboratory animals. Chickens were humanely handled in line with ethical standards laid down in Helniski declaration of 1964 and its later amendment.

### Statistical analysis

All data generated from this experiment were subjected to a two-way analysis of variance in accordance with a completely randomized design using the General Linear Model (GLM) procedure of the Statistical Package for Social Science (SPSS version 16.0). Significant means were separated using Duncan's multiple range test of the same package at ($p<0.05$). The following model was used:

$$Y_{ijk} = \mu + Z_i + S_j + (ZS)_{ij} + E_{ijk} \qquad i = 1\ldots4,\ z;\ j = 1\ldots4,\ s;\ k = 1\ldots.16\ n$$

Where: $Y_{ijk}$ = observation $k$ in level $i$ of factor $Z$ and level $j$ of factor $S$; $\mu$ = qqthe overall mean; $Z_i$ = the effect of level $i$ factor $Z$; $S_j$ = theqq effect of level $j$ factor $S$; $ZS_{ij}$ = the effect of the interaction level i of factor Z with level j of factor S; $E_{ijk}$ = random error with mean 0 and variance $o^2Z$ = number of levels of factor $Z$; e = number of levels of fqactor $S$; n = number of observation for each $Z$ x $S$ combination.

## RESULTS

The results of main effect of dietary nano zinc and selenium supplementation on haematological indices of broiler chickens are presented in Table 2. There were significant ($p>0.05$) differences in haemoglobin, packed cell volume, red blood cells, neutrophils and lymphocytes count. The haemoglobin (Hb) concentration value ranged from 12.05 to 13.35 g/dl. Birds fed 30 and 40 mg/kg NZn supplemented diets had similar ($p>0.05$) but higher ($p<0.05$) haemoglobin values than those on 20 and 50 mg/kg NZn supplemented diets which had similar ($p>0.05$) values. Packed cell volume (PCV) value ranged from 34.78 to 40.01 % and Red blood cells (RBC) value ranged from 5.43 to 6.74 $\times10^9$/l. Neutrophils value ranged from 30.45 to 31.75 % and indicated that birds fed 30 and 40 mg/kg NZn supplemented dies had similar ($p<0.05$) values. However, the value obtained from those on 30 mg/kg NZn supplemented diet had higher ($p<0.05$) neutrophils value than birds fed 20 and 50 mg/kg NZn supplemented diets. The results of lymphocytes mean value ranged from 59.07 to 61.91 % and revealed that birds on 30 and 40 mg/kg NZn supplemented diets had similar ($p>0.05$) values. On the other hand, those birds fed 30 mg/kg NZn supplemented diets had higher ($p<0.05$) value than those on 20 and 50 mg/kg NZn supplemented diets.

The effect of dietary nano selenium showed haemoglobin concentration value ranged from 12.52 to 13.29 g/dl and Packed cells volume value ranged from 37.04 to 40.56 %. Their values revealed that those fed 0.10, 0.15 and 0.25 mg/kg NSe supplemented diets had similar ($p>0.05$) values. Conversely, birds on 0.20 mg/kg NSe supplemented diets had higher ($p<0.05$) Hb and PCV values than those on 0.10, 0.15 and 0.25 mg/kg NSe supplemented diets. Red blood cells value ranged from 5.97 to 6.58 $\times10^9$/l and showed that birds fed 0.20 mg/kg NSe supplemented diet had higher ($P<0.05$) value than those from other treatments.

The results of the effect of dietary nano zinc and selenium supplementation on serum biochemical indices of Arbor acre broiler chickens are presented in Table 3. The results showed that NZn influenced ($p<0.05$) the serum urea, creatinine, nitrogen and alkaline phosphatase

**Table 2.** Effect of dietary nano zinc and selenium supplementation on haematological profile of finished broiler chickens.

| Factors | Hb (g/dl) | PCV (%) | RBC ($\times10^9$/l) | WBC ($\times10^9$/l) | Neutrophils (%) | Lymphocytes (%) | Monocytes (%) | Eosinophils (%) | Basophils (%) | MCV (fl) | MCH (pl) | MCHC (g/dl) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NZn mg/kg | | | | | | | | | | | | |
| 20 | 12.05$^{b}$ | 34.78$^{c}$ | 5.43$^{c}$ | 4.25 | 30.56$^{b}$ | 59.07$^{b}$ | 2.43 | 1.00 | 0.87 | 110.66 | 19.74 | 33.27 |
| 30 | 13.35$^{a}$ | 40.01$^{a}$ | 6.74$^{a}$ | 4.83 | 31.75$^{a}$ | 61.91$^{a}$ | 3.00 | 0.93 | 1.00 | 111.2 | 20.00 | 33.2 |
| 40 | 12.91$^{a}$ | 38.51$^{ab}$ | 6.41$^{ab}$ | 4.44 | 31.09$^{ab}$ | 60.31$^{ab}$ | 2.78 | 1.00 | 0.93 | 112.81 | 20.13 | 33.15 |
| 50 | 12.33$^{b}$ | 38.09$^{b}$ | 6.20$^{b}$ | 4.38 | 30.45$^{b}$ | 59.56$^{b}$ | 2.93 | 1.00 | 1.00 | 110.22 | 20.00 | 33.13 |
| SEM | 0.17 | 0.59 | 0.12 | 0.09 | 0.3 | 0.71 | 0.74 | 0.07 | 0.31 | 0.18 | 0.08 | 0.54 |
| NSe (mg/kg) | | | | | | | | | | | | |
| 0.10 | 12.52$^{b}$ | 37.03$^{b}$ | 5.97$^{b}$ | 4.58 | 31.55 | 59.62 | 2.59 | 1.00 | 0.93 | 112.4 | 19.98 | 33.27 |
| 0.15 | 12.53$^{b}$ | 37.04$^{b}$ | 6.10$^{ab}$ | 4.3 | 30.92 | 60.03 | 2.81 | 1.00 | 0.93 | 109.44 | 19.94 | 33.22 |
| 0.20 | 13.29$^{a}$ | 40.56$^{a}$ | 6.58$^{a}$ | 4.64 | 30.53 | 61.38 | 3.00 | 1.00 | 0.93 | 110.65 | 20.01 | 33.09 |
| 0.25 | 12.30$^{b}$ | 36.75$^{b}$ | 6.13$^{ab}$ | 4.38 | 30.85 | 59.62 | 2.75 | 0.93 | 1 | 109.41 | 19.94 | 33.16 |
| SEM | 0.17 | 0.59 | 0.12 | 0.09 | 0.3 | 0.71 | 0.74 | 0.07 | 0.31 | 0.18 | 0.08 | 0.54 |
| Main effects and Interaction | | | | | | | | | | | | |
| NZn | 0.01 | 0.01 | 0.01 | 0.07 | 0.03 | 0.04 | 0.07 | 0.4 | 0.31 | 0.05 | 0.12 | 0.23 |
| NSe | 0.01 | 0.01 | 0.01 | 0.06 | 0.08 | 0.13 | 0.06 | 0.4 | 0.8 | 0.76 | 0.92 | 0.1 |
| NZnxNSe | 0.01 | 0.15 | 0.01 | 0.09 | 0.11 | 0.21 | 0.08 | 0.12 | 0.11 | 0.49 | 0.08 | 0.06 |
| NR | 7-13.5 | 22– 45 | 4 – 6 | 4 – 10 | 30 – 70 | 28 – 72 | 0 – 3 | 0 – 1 | 0 – 1 | 90-140 | 33-47 | 30-36 |

abc = means in the row carrying different superscripts differs significantly ($p<0.05$). Hb = Haemaglobin, PCV = Packed cell volume, RBC = Red blood cells, WBC = White blood cells, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin and MCHC = Mean corpuscular haemoglobin concentration. NZn = Nano Zinc, NSe = Nano Selenium, NR = normal range: Bounous and Stedman (2000).

**Table 3.** Effect of dietary nano zinc and selenium supplementation on serum biochemical profile of broiler chickens.

| Factors | Glucose (mmol/dl) | Urea (mmol/dl) | Creatinine (u/l) | Cholesterol (mmol/dl) | T. protein (g/dl) | Albumin (g/dl) | Nitrogen (mmol/dl) | AST (u/l) | ALT (u/l) | ALP (u/l) |
|---|---|---|---|---|---|---|---|---|---|---|
| NZn (mg/kg) | | | | | | | | | | |
| 20 | 4.59 | 5.81$^{b}$ | 5.68$^{b}$ | 91.42 | 3.99 | 1.68 | 4.45$^{c}$ | 86.91 | 3.79 | 622.87$^{ab}$ |
| 30 | 5.41 | 6.80$^{a}$ | 6.56$^{a}$ | 92.17 | 3.90 | 1.98 | 4.15$^{c}$ | 81.85 | 3.54 | 630.94$^{ab}$ |
| 40 | 5.04 | 5.95$^{b}$ | 6.62$^{a}$ | 91.19 | 3.37 | 1.60 | 5.08$^{b}$ | 86.90 | 3.06 | 681.92$^{a}$ |
| 50 | 5.42 | 6.51$^{ab}$ | 6.50$^{ab}$ | 91.62 | 3.91 | 1.58 | 5.66$^{a}$ | 85.18 | 3.26 | 613.14$^{b}$ |
| SEM | 0.24 | 0.24 | 0.19 | 1.99 | 0.27 | 0.15 | 0.13 | 2.43 | 20.68 | 0.17 |
| NSe (mg/kg) | | | | | | | | | | |
| 0.10 | 4.77$^{b}$ | 6.48$^{a}$ | 6.31$^{ab}$ | 92.57 | 3.89 | 1.53 | 4.49 | 84.75 | 3.39 | 623.33 |
| 0.15 | 4.56$^{b}$ | 5.68$^{b}$ | 6.18$^{ab}$ | 91.82 | 4.05 | 1.77 | 4.89 | 86.75 | 3.32 | 630.37 |
| 0.20 | 5.91$^{a}$ | 6.23$^{ab}$ | 5.87$^{b}$ | 92.78 | 3.41 | 1.67 | 4.94 | 84.52 | 3.48 | 631.06 |
| 0.25 | 5.21$^{ab}$ | 6.68$^{a}$ | 7.00$^{a}$ | 89.22 | 3.82 | 1.88 | 5.03 | 84.85 | 3.45 | 664.10 |
| SEM | 0.24 | 0.24 | 0.28 | 1.99 | 0.27 | 0.15 | 0.13 | 2.43 | 20.68 | 0.17 |
| Main effects and Interaction | | | | | | | | | | |
| NZn | 0.06 | 0.01 | 0.02 | 0.37 | 0.24 | 0.98 | 0.03 | 0.42 | 0.06 | 0.02 |
| NSe | 0.01 | 0.04 | 0.01 | 0.57 | 0.40 | 0.43 | 0.19 | 0.91 | 0.92 | 0.51 |
| NZn x NSe | 0.02 | 0.01 | 0.02 | 0.14 | 0.62 | 0.09 | 0.02 | 0.09 | 0.83 | 0.01 |
| NR | 2.8-8.9 | 3.3-14.5 | 0.1-14 | 87-192 | 3.0-4.9 | 1.17-2.74 | 2.5-7.1 | 70-220 | 6-36 | 568-883 |

$^{abc}$ = means in the same row carrying different superscript varied significantly ($p<0.05$). SEM = standard error of mean, P–value = probability levels, mg = milligram and g/b = gram per bird, NZn = Nano Zinc and NSe = Nano Selenium, NR = normal range: Catherine (1985). AST = Aspartate amino transaminase, ALT = Alanine amino transaminase, and ALP = Alkaline Phosphatase.

**Table 4.** Effect of nano zinc and selenium enriched diets on relative weight of immune organs and serum immunoglobulins of broiler chickens.

| Factors | Thymus (g/b) | Bursa of fabricius (g/b) | Spleen (g/b) | IgG (g/l) | IgA (g/l) | IgM (g/l) |
|---|---|---|---|---|---|---|
| NZn (mg/kg) | | | | | | |
| 20 | 2.58$^{b}$ | 1.82$^{c}$ | 1.43$^{c}$ | 4.09 | 2.42 | 2.01$^{b}$ |
| 30 | 2.82$^{b}$ | 2.09$^{ab}$ | 1.79$^{b}$ | 4.21 | 2.61 | 2.16$^{ab}$ |
| 40 | 2.67$^{b}$ | 1.95$^{bc}$ | 1.74$^{b}$ | 4.49 | 2.55 | 2.27$^{a}$ |
| 50 | 3.28$^{a}$ | 2.21$^{a}$ | 2.00$^{a}$ | 4.13 | 2.45 | 2.29$^{a}$ |
| SEM | 0.13 | 0.56 | 0.05 | 0.15 | 0.11 | 0.06 |
| NSe (mg/kg) | | | | | | |
| 0.10 | 2.94 | 2.16$^{a}$ | 1.67 | 4.21$^{ab}$ | 2.61$^{ab}$ | 2.04$^{b}$ |
| 0.15 | 2.98 | 2.16$^{a}$ | 1.78 | 3.87$^{b}$ | 2.31$^{b}$ | 2.19$^{b}$ |
| 0.20 | 2.73 | 1.80$^{b}$ | 1.71 | 4.31$^{ab}$ | 2.35$^{b}$ | 2.11$^{b}$ |
| 0.25 | 2.7 | 1.95$^{b}$ | 1.79 | 4.53$^{a}$ | 2.77$^{a}$ | 2.39$^{a}$ |
| SEM | 0.13 | 0.56 | 0.05 | 0.15 | 0.11 | 0.06 |
| Main effects and Interaction | | | | | | |
| NZn | 0.02 | 0.01 | 0.01 | 0.28 | 0.68 | 0.02 |
| NSe | 0.34 | 0.02 | 0.24 | 0.04 | 0.01 | 0.01 |
| NZn x NSe | 0.01 | 0.11 | 0.84 | 0.03 | 0.01 | 0.01 |
| Normal range | None | None | None | 1 - 13.5 | 0.31 – 4 | 0.71 – 2.5 |

abc = means in the same row carrying different superscript varied significantly (P<0.05). SEM = standard error of mean, P–value = probability levels, mg = milligram and g/b = gram per bird, NZn = Nano Zinc and NSe = Nano Selenium, NR = normal range: Catherine (1985). IgG = Immunoglobulin G, IgA = Immunoglobulin A, and IgM Immunoglobulin M.

of Arbor acre broiler chickens. Urea concentration values ranged from 5.81 to 6.80 mmol/dl. It was revealed that birds fed a 30 mg/kg NZn-supplemented diet had greater (p<0.05) urea concentration values than those on 20 and 40 mg/kg NZn-supplemented diets.

Creatinine values ranged from 5.68 to 6.62 u/l, the results indicated that birds on 30 and 40 mg/kg NZn supplemented diets had higher (p<0.05) values than those on 20 mg/kg NZn supplemented diets.

The findings of nitrogen value ranged from 4.15 to 5.66 mmol/dl and demonstrated that fed 50 mg/kg NZn supplemented diet had (p<0.05) higher value than those on 20, 30 and 40 mg/kg NZn supplemented diets. Alkaline phosphatase (ALP) value ranged from 613.14 to 681.92 u/l. The results revealed birds on a 40 mg/kg NZn-supplemented diet had higher (p<0.05) ALP values than birds on a 50 mg/kg NZn-supplemented diet.

The effect of nano selenium supplementation (NSe) showed all the serum biochemical parameters measured were not (p>0.05) significantly affected except glucose concentration, urea and creatinine. The glucose value ranged from 4.56 to 5.21 mmol/dl. The value obtained from those on 0.20 mg/kg NSe supplemented diet was significantly (p<0.05) higher than birds fed 0.10 and 0.15 mg/kg NSe supplemented diets, but not different (p>0.05) from those birds on 0.25 mg/kg NSe supplemented diet.

The results of urea value ranged from 5.68 to 6.68 mmol/dl which indicated birds on 0.10 and 0.25 mg/kg NSe supplemented diets had higher (p<0.05) values than those fed 0.15 mg/kg NSe supplemented diet.

Creatinine values ranged from 5.87 to 7.00 u/l and revealed that those on 0.10, 0.15 and 0.25 mg/kg NSe supplemented diets had (p>0.05) similar values. Conversely, birds fed 0.25 mg/kg NSe supplemented diets had (p<0.05) higher values than birds on 0.20 mg/kg NSe supplemented diet amongst the treatments.

The results of the effect of dietary nano zinc and selenium-enriched diets on the relative weight of immune organs and serum immunoglobulins of broiler chickens are shown in Table 4. The effects of nano zinc enriched diets revealed that thymus, bursa of Fabricius, spleen and IgM values were significantly (p<0.05) affected by nano zinc levels. Thymus weight of birds fed 50 mg/kg NZn-supplemented diets was (p<0.05) higher than those fed 20, 30 and 40 mg/kg NZn-supplemented diets. The values of the bursa of Fabricius showed birds fed 50 mg/kg NZn-supplemented diets had higher (p<0.05) value (2.21g/b) than birds fed 20 (1.82 g/b) and 40 (1.95 g/b) mg/kg NZn-supplemented diets.

Spleen weight of birds fed 50 mg/kg NZn-supplemented diets was greater (p<0.05) in value than those fed 20, 30 and 40 mg/kg NZn-supplemented diets. The values of IgM indicated that birds fed 40 and 50 mg/kg NZn-supplemented diets had higher (p<0.05) values than those on 20 mg/kg NZn-supplemented diets.

The results of nano selenium (NSe) enriched diets on the relative weights of immune organs and serum immunoglobulins showed that the parameters measured were significantly (P<0.05) influenced except for thymus and spleen. The results of the bursa of Fabricius demon-

strated birds fed 0.10 and 0.15 mg/kg NSe-supplemented diets had higher ($p<0.05$) bursa of Fabricius values than those birds fed 0.20 and 0.25 mg/kg NSe-supplemented diets. Values of IgG revealed that birds fed 0.25 mg/kg NSe-supplemented diets had higher ($p<0.05$) than those on 0.15 mg/kg NSe-supplemented diets.

The values of IgA reflected birds fed 0.25 mg/kg NSe-supplemented diet had greater IgA ($p<0.05$) values than those fed 0.15 and 0.20 mg/kg NSe-supplemented diets. IgM values indicated that birds on 0.25 mg/kg NSe-supplemented diets had higher ($p<0.05$) values than those on 0.10, 0.15 and 0.20 mg/kg NSe-supplemented diets respectively.

## DISCUSSION

Limited studies exist on the combination of ano zinc and selenium in the nutrition of Arbor acre broiler chickens. The values obtained in the present study for haemotological parameters fall within the normal range values reported by Bounous and Stedman (2000) for healthy birds. The increase in Hb, PCV and RBC concentrations of this study may be associated with the fact that nano zinc has some innate quality to induce the production of erythrocytes which correlate with the normal functioning of the bone marrow in birds as reported by Iyaode *et al*. (2020). However, a higher inclusion level of zinc could lead to decreased concentration of Hb, PCV and RBC as demonstrated in the present data. Thus, it is an indication that optimum levels of zinc needed by Arbor acre broiler chickens were attained at 40 mg/kg NZn supplemented diets. Alabi *et al*. (2014) reported that in a dose-related study, as the test ingredients increase to a certain point, the parameter of interest continues to increase until a point of equilibrium or optimal level is reached; thereafter, any increment in the test ingredients will lead to decrease as observed in Hb, PCV and RBC values of the present study. The present study confirmed the recommendation of NRC (1994) which established that 40 mg/kg of zinc can satisfy the requirement of broiler chickens (Sahoo *et al*., 2014a). This is in line with the result of Fawzy *et al*. (2016) who indicated increased haemoglobin, PCV and RBC concentration in broiler chickens fed diets supplemented with different levels of zinc oxide.

Similarly, these results are also comparable to the reports of Raina *et al*. (2018) who supplemented zinc to broiler diets; Odunitan-Wayas *et al*. (2018) who fed Ovambo chickens provitamin A bio-fortified maize and Iyaode *et al*. (2020) who fed Cobbs 500 and Arbor-acre broilers strain ginger supplemented diets. However, these findings contradict the report of Mohammed *et al*. (2018) who stated dietary supplementation of zinc in the diet of broiler chickens did not influence Hb, PCV and RBC counts. The reason for the disparity might be due to variations in the test ingredients and environments. The dietary addition of NSe at 0.20 mg/kg resulted in higher haemoglobin, PCV and RBC concentrations when compared with other groups. In this study, the incorporation of NSe beyond 0.20 mg/kg revealed a decrease in the values of these parameters. This might be an indication that 0.20 mg/kg of NSe supplementation was adequate for these parameters. It implies that higher doses of nano selenium inclusion may lead to a reduction in the concentration of haemoglobin, PCV and RBC in the blood of broiler chicken and a waste of resources. Furthermore, the antioxidant property of selenium nanoparticles in protecting the red blood cells and erthyrocyte membrane against ROS oxidation may be the reason for a significant increase in the blood profile of the birds.

In addition, haemoglobin, PCV and RBC values obtained from dietary supplementation of nano selenium are within the normal range as reported by Bounous and Stedman (2000). The present results are similar to the report of Jamima *et al*. (2020) who documented that the inclusion of nano Se in the diets of broiler chickens significantly increased haemoglobin (Hb), red blood cells (RBC) and packed cell volume (PCV) while white blood cells were not significant when compared to the control group. Similarly, while previous reports have demonstrated a significant difference, Mohapatra *et al*. (2014) reported an increase in the concentration of Hb, PCV and RBC from the dietary addition of nano Se to broiler chickens. Furthermore, Tayeb *et al*. (2012) also observed increased Hb, PCV and RBC counts from feeding broiler chickens with Se and vitamin E-supplemented diets.

On the contrary, the results disagreed with the observation of Chen *et al*. (2014) and Boostani *et al*. (2015) who reported no significant variation in the h/laemoglobin concentration of broiler chickens fed diets supplemented with nano selenium. The demonstration of dissimilarity in different results might depend on climatic factors, as most referenced values were established in temperate regions whose data may not match tropical animal characteristics due to the differences in environmental conditions as well as genetic variability (Onunkwo *et al*., 2018).

The blood profile of Arbor acre broiler chickens demonstrated the complimentary interactive effects of combined dietary supplementation of zinc and selenium on Hb, PCV, RBC, Neutrophils and Lymphocytes. This improvement could be attributed to the bioactive role of these elements in regulating nutrient metabolism, oxidation and defence system leading to better utilization for proper growth and health as reported by Sahoo *et al*. (2014b) and Jamima *et al*. (2020).

Serum biochemistry parameters evaluated are important as they provide valuable information about the health and immune status of the animals. The serum parameters are good indicators for the physiological conditions of the animal body and any deviation in the serum indices is important in assessing the response of such animals to various physiological perturbations (Khan and Zafar, 2005).

Dietary supplementation of nano zinc to broiler chickens significantly improved urea, creatinine, nitrogen and ALP

values which agree with the report of Iyaode *et al*. (2020) for international reference range values of serum biochemical indices for healthy broiler chickens. This could mean that NZn and Se can supply and improve nutrient utilization, antioxidant functionality and immune modulating capacity of chickens as observed by Yang *et al*. (2016). These results agree with the findings of other studies where minerals were supplemented separately and/or in combination as reported by Kaminski *et al*. (2014), Fawzy *et al*. (2016) and Raina *et al*. (2018).

Similarly, the results obtained in this study are in line with what was observed by Hafez *et al*. (2017) who evaluated the effect of zinc oxide nanoparticles on the performance and absorptive capacity of the intestinal villi in broiler chickens. The author detected a positive influence on the serum urea and creatinine. On the contrary, the present data is not in agreement with a previous report that the dietary addition of nano zinc oxide did not influence serum urea and creatinine concentration of commercial broiler chickens (Hatab *et al*., 2022). The discrepancies of the current result with other reports might be due to breed differences and inclusion levels. This implies that 30 mg/kg NZn could be adequate for good health as the value obtained in the present study falls within reference values reported for healthy birds (Meluzzi *et al*., 1992).

Alkaline phosphatase is an enzyme responsible for dephosphorylation of a substrate which is produced in entire body tissues and activated in alkaline pH. Thus, higher levels of ALP can be mostly seen in liver damage Senanayake *et al*. (2015). Dietary addition of 40 mg/kg nano-ZnO to Arbor acre broiler chickens significantly increased serum ALP activity than the other groups, suggesting that NZn in the intestine, kidneys and bones influences and increases absorption of calcium into the extracellular fluid and promotes the formation of ALP in the epithelial cells (Guyton and Hall, 2006). In addition, increased ALP activity may be attributed to enhanced concentrations of cholesterol. This result is similar to the reports of Usama *et al* (2020) who clarified that dietary inclusion of 40 ppm ZNOPs significantly increased the birds' serum ALP.

Supplementation of 0.25 mg/kg NSe in the diet of broiler chickens demonstrated higher concentrations of urea and creatinine than other groups. This might imply that the effect of a 0.25 mg/kg NSe-supplemented diet is adequate for the maintenance of normal body physiological processes in *Arbor acre* broiler birds. The addition of nano Se in the diet of broiler chickens revealed irregular patterns across the treatment levels. The values recorded from urea and creatinine are an indication that the nano zinc and selenium did not impair kidney functioning. The present study corresponds with the results of Hassan *et al*. (2020) who reported a significant difference in the amount of urea and creatinine produced by broiler chickens fed NSe-supplemented diets. However, this data disagreed with the previous reports of Salim *et al*. (2015) and Ibrahim *et al*. (2019) who reported that increasing supplemental Se level from 0.3 to 0.45 ppm in broiler diets did not cause any significant difference in serum urea and creatinine level. The reason for the differences with the current study could be due to nano Se inclusion levels and breed differences of broiler chickens used by Ibrahim *et al*. (2019).

Liver enzymes are clinically used as biomarkers for liver health and diseases involving other organs such as the heart and kidneys. An increase in these enzymes envisages liver damage and leakage of the hepatic enzymes into the blood may have occurred, whereas reduced levels usually indicate healthy livers. However, low levels could still show some liver damage. Additionally, the increase in blood glucose in the 50 mg/kg NZn with 0.20 mg/kg NSe compared to other treatments suggests zinc and selenium bioactivity in the breaking down of nutrients leading to more glucose absorption and transforming to muscle fibre as confirmed by body weight gain. These results support the reports of several authors Kaminski *et al*. (2014), Fawzy *et al*. (2016) and Raina *et al*. (2018) that documented similar trends in their studies.

The bursa of fabricius is the main lymphoid organ in broiler chickens that play an important function in B lymphocyte differentiation (Sahoo *et al*., 2014a). The dietary enriched diets of nano zinc significantly improved the relative weights of immune organs and immunoglobulins of Arbor acre broiler chickens. This might be due to the biological functionality of nano Zn in mitigating the negative effects of heat stress by maximizing the antioxidant defence system and minimizing lipid peroxidation in the blood plasma. This translates into enhanced immunity and disease resistance in broiler chickens. Higher inclusion levels of both NZn and NSe have led to the higher thymus, bursa of Fabricius, spleen and IgM of the Arbor acre broiler chickens. This might mean that the immunity of this breed of chickens was enhanced at a higher dose of NZn and NSe. This finding agreed with the previous observation of Sahoo *et al.* (2014a) who found that 15 ppm Zn-Met. and 0.06 ppm NZnO significantly enhanced the relative weights of the spleen, bursa of Fabricius, and thymus in comparison with the rest of the treatments.

These results partially agreed with the observation of El-Katcha *et al.* (2017) who documented that replacement of dietary inorganic ZnO with Zn nanoparticles significantly improved the relative weight of thymus and spleen. In the same vein, Bakhshalinejad *et al*. (2018) also indicated that the dietary addition of zinc nanoparticles increases the activity of antioxidant enzymes and reduces lipid peroxidation in poultry raised under heat-stress conditions.

This could have explained why broilers chicken fed dietary organic or nano zinc increased thymulin activity; thus, enhancing the immune response through increased maturation of T-lymphocytes and activation of B lymphocytes by T-helper cells (Hudson *et al.,* 2004). Furthermore, Mohammadi *et al.* (2015) revealed spleen and bursa of Fabricius (%) of broiler chicks were significantly higher when the diet was supplemented with

40 mg/kg of Zn nano compared with the control. The current results are similar to those of Chand *et al*. (2014) and Abudabos *et al*. (2017) who reported significant improvement in the relative weight of immune organs of broiler chickens fed a diet supplemented with nano zinc. On the other hand, these findings disagreed with reports by Donmez *et al*. (2001) where Zn enriched diets had no effect on peripheral blood leukocyte counts and serum lymphocyte concentrations.

Furthermore, dietary additions of nano zinc affect serum immunoglobulins IgM concentration of Arbor acre broiler chickens. This may be attributed to the bioactivity of zinc in boosting immune systems leading to higher production of interleukin-2 against foreign bodies. This data is in harmony with those obtained by Soni *et al*. (2013) who concluded that cellular immunity and antibody production of broiler breeders was significantly improved by dietary addition with organic zinc.

Arbor broiler chickens fed dietary nano selenium at the rate of 0.25 mg/kg diet affect serum concentration of immunoglobulins IgA, IgG and IgM. These glycoprotein molecules are produced by plasma cells (white blood cells). Thus, acts as a critical part of the immune response by recognising and binding to particular antigens that invade the body such as bacteria or virus aiding in their destruction. This might imply that when the birds are not infected by pathogens, the disease will not illicit its production. Selenium is known to influence the immune response of broiler chickens and when administered in nano form increased absorption and transport Sahoo *et al* 2014b). These are in agreement with those obtained by Dalia *et al*. (2017) who also reported a significant difference in the serum IgA, IgG and IgM concentration of broiler chickens fed organic selenium. However, the present results disagreed with the observation of Cai *et al*. (2012) who indicated no significant effect in serum immunoglobulins of broiler chickens fed dietary nano selenium. The variability of current results with other findings may be due to a higher dose of nano selenium-enriched in the diets fed to broiler chickens.

Dietary addition of nano zinc and selenium to Arbor acre broiler chickens had an interaction effect on the relative weights of immune organs which were within the normal range values reported by Catherine (1985) who declared that 1-13.5 (g/l) IgG, 0.31- 4 (g/l) IgA and 0.71 – 2.5 (g/l) IgM were adequate to confer normal antioxidant and immune defence system without compromising the general wellbeing of the broiler chickens. These results agreed with Zhang *et al*. (2012) who reported that broiler chicks fed a diet supplemented with selenium showed significant improvement in immunological parameters.

## Conclusion and Recommendation

It can be concluded that supplementing the diets of Arbor Acre broiler chickens with nano zinc (30 mg/kg) and selenium (0.20 mg/kg) from 1 to 49 days of age improved haematological parameters such as haemoglobin, packed cells volume, red blood cells, neutrophils and lymphocytes. Moreover, the serum biochemical profile of the birds fed diets supplemented with nano zinc and selenium at 30 mg/kg NZn with 0.25 mg/kg NSe had a profound impact on glucose, urea, creatinine and alkaline phosphatase levels. Higher doses of nano zinc (up to 50 mg/kg) and selenium (0.10-0.25 mg/kg) were found to enhance immune organ development, including thymus, bursa of Fabricius, and spleen, as well as immunoglobulin levels (IgA, IgG, and IgM).

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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