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Hematological and Biochemical Performance of Poultry Following Zinc Oxide and Sodium Selenite Supplementation as Food Additives

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Abstract

The present work was performed to study the effect of zinc oxide and sodium selenite alone or in combination on the growth performance of poultry when added to the ration as feed additives. One hundred and sixty of 1-d- old broiler chicken were used and divided equally into four groups each of 40 chicks as follow; basal diet supplemented group [1], zinc oxide (2 g /10 kg ration for 4 weeks) supplemented group [2], sodium selenite (15.4 mg / 10kg ration for 4 weeks) supplemented group [3] and combination group [4]. Each week from starting the experiment till the fourth week, blood samples and liver tissue were collected for assessment of selective hematological, biochemical and antioxidant enzymes activities of birds. The results achieved that zinc oxide and sodium selenite in combination had significant influence on broiler chicken antioxidant status, hematopoietic system responsibility and growth performance and did not induced hepatic and renal toxicity. In conclusion, zinc oxide and sodium selenite when used together with the presently used doses were powerful antioxidant nontoxic feed additives for poultry.

INTRODUCTION

Poultry industry is an important economical business in the agriculture field, providing meat and eggs that increase the nutritional quality of human food. It has become an attractive business due to its rapid outcomes [1]. The overall economy of a broiler is assessed by its productivity and growth performance [2]. The first role of feed is to cover the metabolic needs of the bird. Presence of high quality feed at an equitable pricing is one of the limiting factors in the business success. Feed cost comprises 60 to 65% of the total broiler production costs [1]. Recently, the feed commerce is focused on the usage of trace elements with immunostimulant and antioxidant properties as alternative feed additives.

The trace mineral zinc (Zn) is a key element in the broiler poultry development [3] and involved in many physiological, metabolic and digestive processes in the body as it acts as a cofactor of more than 300 enzymes which essential for reproduction, growth, protein and carbohydrate metabolism. In poultry, zinc is essential for reproductive system development [4]. Moreover it is responsible for activation of the antioxidant status [5] and immune system of the bird indirectly by interaction with growth and infectivity of organisms that are pathogens to animals and directly by increasing the thymocytes and peripheral T cells counts with stimulation of natural killer cells [6]. Also it accelerates the neutrophils and antibodies production and improves the macrophage functions [7].

Selenium (Se) is an essential mineral in poultry feeding for the maintenance of optimal health and meat quality [8] because it is involved in the antioxidant system of the body through the regulation of the antioxidant defense mechanism in all living tissues by controlling the body's glutathione (GSH) pool and its major Se-containing antioxidant enzymes, glutathione peroxidase

(GPx) and thioredoxin reductase [9] which are free radical scavengers and protect the cells membrane phospholipids from lipid peroxidation (LP) [10]. With this in mind, the aim of the present study was to verify the following hypothesis: dietary supplementation with zinc and selenium for poultry, improves the growth performance, antioxidative and hematopoietic and immunological status of the birds.

MATERIALS AND METHODS

Tested feed additives

Zinc oxide was obtained from Pure Laboratory Chemicals, El-Nasr Pharmaceutical chemicals Co., Egypt. Sodium selenite was purchased from Laboratory reagents, Ltd, Poole, England.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Chicks

One hundred and sixty 1-d- old commercial broiler chicks (Hubbard strain) were purchased from Al-Kahira Poultry Company to be used for this experiment. Chicks were kept under standard hygienic conditions. The chicks were wing-banded individually and reared under uniform management care in stainless steel battery brooders (60 cm × 75 cm × 45 cm for 5 chicks) with raised wire floor in an open-sided house. They were provided with a rearing temperature of 34 ± 1°C up to 7 d of age and gradually reduced to 26 ± 1°C by 21 day of age after which no supportive heat was provided. The relative humidity during the experimental rearing ranged from 67 to 77 %. The chicks were vaccinated against Marek's disease, Newcastle disease, and infectious bursal disease following the prescribed schedule (Table 1). A commercial diet (Table 2) and water were provided ad-libitum throughout the experimental period.

Experimental grouping and sampling

Broiler chicks were assigned to four equal groups containing 40 chicks, each group divided into 4 replicates of 10 birds. The groups are basal diet supplemented group which kept as control (C), Zinc group (Zn) which supplemented with zinc oxide (2g/10 kg ration), selenium group (Se) which supplied with sodium selenite (15.4 mg / 10kg ration) and combination group (Zn+Se) which supplied with both zinc oxide and sodium selenite by the same doses.

Table 1. Vaccination schedule of the broiler chicks.

Type of vaccine	Company	Route of vaccination	Age of vaccination / day
Influenza	Intervet company	Injection	1
Hitchner B1+IP	Iso company	Eye drop	4
Lasota	Intervet company	Eye drop	14
D78	Intervet company	Eye drop	18
Lasota	Intervet company	Eye drop	24

Table 2: Nutrient composition (%), ingredient, vitamins and minerals of the basal diet (%).

Nutrient composition (%)	**Quantity**
Crude protein	22.05
Metabolizable energy (kcal/kg)	2,878
Lys	1.19
Calcium	1.09
Met	0.50
Nonphytate phosphorus	0.45
Ingredient	
Yellow maize	59.55
Soybean meal	35.00
Di calcium phosphate	1.70
Shell grit	1.80
Common salt	0.50
Choline chloride	0.26
DL Methionine	0.26
Vitamins	0.04
Trace minerals	0.10
Coccidiostat	0.05
Antibiotics	0.05
Starch	0.69
Vitamins	
Vitamin A	16,500 IU
Vitamin D3	3,150 ICU
Vitamin E	12 mg
Vitamin K	2 mg
VitaminnB1	1.2 mg
Vitamin B2	10 mg
Vitamin B6	2.4 mg
Vitamin B12	12 µg
Niacin	18 mg
Pantothenic acid	12 mg
Minerals	
Mn	60 mg
Fe	60 mg
Cu	10 mg
I	1.2 mg

Eight broiler chicks, two from each replicate (2×4) at all tested groups were taken for blood collection from wing vein at the end of 1st, 2nd, 3rd and 4th week from starting the experiment. The 1st blood sample was collected in an EDTA tube for hematological analysis (Eryhtrogram and Leukogram), while the 2nd blood sample was collected without anticoagulant into a clean dry centrifuge tube, left to clot at room temperature then centrifuged at 3000 rpm for 5 min for serum separation to perform the clinico-biochemical tests. After that, they were slaughtered for liver tissue collection which homogenized to assess the glutathione peroxidase and superoxide dismutase activities. Birds were

weighted before blood sampling at the end of the 4th week and internal organs (liver, kidney, heart, gizzard, spleen, thymus and bursa) weights were recorded after slaughtering.

Hematological studies

Erythrocytic and leukocytic counts were performed using an improved Neubauer hemocytometer and Natt and Herrick solution as a special diluent for chicken's blood according to [11]. The packed cell volume was estimated by micro hematocrit centrifuge according to [12]. Hemoglobin estimation was performed using the cyanomethemoglobin colorimetric method after centrifugation according to [13]. Mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) were calculated. Blood films were made, fixed by methyl alcohol, stained by Giemsa stain for differential leukocytic count and detection of abnormalities in RBCs morphology according to [14].

CLINICO-BIOCHEMICAL TESTS

Estimation of serum zinc and selenium

Serum zinc and selenium concentrations were determined by the atomic absorption technique which utilized using a Buck scientific 210VGP Atomic Absorption Spectrophotometer at the central lab of Faculty of Veterinary, Zagazig University. The registered values for zinc and selenium were expressed as mg/g wet weight (ppm).

Antioxidant enzyme activities

Liver samples were homogenized before analysis with a nine-fold amount of physiological saline using electrical homogenizer at 3000 r.p.m for 15 minutes and the supernatant fraction was used for determination of enzyme activity [15] SOD and GPX activities were measured by the methods previously mentioned by *Kakkar et al.* and *Paglia et al.* [16, 17].

Organs functions investigation

All biochemical tests were performed calorimetrically using commercial kits of Diamond-Diagnostic, ELITech, BioMerieux and Spinreact Companies by Semi-automated Photometer 5010 V5+ (RIELE GmbH & Co, Berlin, Germany).Serum activities of Aspartate Transaminase (AST) and Alanine Transaminase (ALT) were measured by the method of Reitman and Frankel [18] while, alkaline phosphatase (ALP) activity was estimated by using the methods of Kind and King [19]. Uric acid and creatinine were estimated using diagnostic kits [20,21].

Protein profile assay

Serum total proteins, albumin levels were determined according to Henry et al. and Doumas et al. [22,23].Values of globulin (G) were calculated by subtracting the value of albumin from the total protein whereas A/G ratio was calculated according to results of albumin and globulin.

Lipid profile assay

Total cholesterol, Triglycerides, HDL-C and glucose were estimated using colorimeteric diagnostic kits [24-27]

Statistical analysis

Data were analyzed by means of one way (ANOVA) using the software statistical program (SPSS, ver.16.00, USA). Data are expressed as the mean ± SE, and results were statistically significant at $P \leq 0.05$ [28].

RESULTS AND DISCUSSION

Zinc addition to poultry chick's basal diet caused a significant ($P \leq 0.05$) increase on body weight (Table 3). This was agreement with the findings of Burrell et al. [29] who found that poultry diet containing 30 ppm of Zn was sufficient to obtain optimum performance during the initial 3 weeks of age. Meanwhile, no difference was observed in body weights between groups fed diets with or without supplemental Zn [30]. The weights of lymphoid organs as spleen, thymus and bursa were increased by the supplemented Zn because it supports better humoral and cell-mediated responses. These findings were similar to those observed by Sunder et al. [31] and disagreed with El-Kaiaty et al. [32] who found that Zn-methionine had no effect on bursa, thymus and spleen relative weights at six weeks of age for Fayoumy breed. Also, Osman and Ragab [33] reported that supplementation of broiler chicks with Zn did not affect the relative weights of bursa, spleen and thymus. *Namra et al*. [34] reported that quails supplied with Zn 50 mg/Kg diet, in organic and inorganic forms, had no effect on the relative weights of spleen and bursa. We are not forgetting that Se also improved the growth rate of broilers chicks which could be related to the increased concentrations of the active form of thyroid hormone in the serum of chicks supplemented with organic Se as well as to the immunomodulating properties of selenium [35].The similar results were obtained by Ibrahim et al. [36] who reported that chicken reared under the heat stress fed diet supplemented with Se showed an improvement in the body weight and feed efficiency. Similarly *Biswas et al.* [37] found an increase in the body weight in broiler chicks supplemented with selenium at a dose of 0.5- 1 mg/kg diet .Also, increased feed efficiency and body weight gain is reported in broilers chicken fed diet supplemented with Se-enriched yeast [38]. On the contrary, it has been reported that Se supplementation revealed no effects on body weight gain and feed efficiency [39] in broilers. Zn and Se in combination had a positive effect on growth performance of birds, which may be attributed to the ability of both elements to improve the nutrient digestion through their protective effect on pancreatic tissue against oxidative damage that help the pancreas to secrete digestive enzymes properly [40].

Concerning with the serum zinc and selenium concentration analysis results, significant ($P \leq 0.05$) increase in serum zinc concentration in chicks treated with zinc alone and in combination with selenium was found, while serum selenium concentration was increased in chicks treated with selenium alone or in combination with zinc following one, two, three and four weeks from starting the treatment (Table 4).Superoxide dismutase is the major antioxidant enzyme containing copper, zinc, and manganese [41], which catalyzes the dismutation of superoxide anions and facilitates the conversion of toxic superoxide anions into nontoxic molecular oxygen [42].Our results (Table 5) agreed with the previous studies which confirmed the role of Cu/Zn-SOD in cellular protection by reducing the production of lipid peroxidation and superoxide anions [43]. But these findings are not in accordance with the result of *Aksu et al.* [44] who found no significant changes in liver SOD in Zn supplemented chicks.

Table 3: Effect of oral supplementation with zinc oxide (2 gm/10 kg diet), sodium selenite (15.4 mg/10 kg diet) and their combination on body (kg), liver, kidney, gizzard, heart, spleen, thymus and bursa (g) weights along the experimental periods.

	4th week							
	Body wt	**Liver**	**Kidney**	**Gizzard**	**Heart**	**Spleen**	**Thymus**	**Bursa**
C	1.40±.04c	34.38±1.12b	2.42±.18c	21.83±.90c	6.28±.18d	1.02±.02c	2.70±.18c	1.17±.07c
Zn	1.46±.01bc	35.82±1.22b	3.79±.29b	22.46±1.05c	8.60±.28c	2.11±.22b	2.73±.37c	1.35±.13c
Se	1.49±.01ab	42.66±.98a	4.11±.29b	32.38±.86b	11.56±.35b	2.50±.10b	4.24±.37b	2.91±.12b
Zn + Se	1.56±.02a	45.32±1.59a	6.14±.44a	35.78±.96a	14.22±.39a	4.03±.19a	5.91±.18a	5.22±.27a
LSD	0.08	3.63	0.93	2.75	0.91	0.46	0.85	

Each value represents the mean of 8 birds ± S.E. All data having different letters are differing significantly at p ≤0.05. L S D: Least significant difference. control (C), zinc (Zn) supplemented ,selenium (Se) supplemented and combination (Zn+Se) groups

Table 4: Effect of oral supplementation with zinc oxide (2 gm/10 kg diet), sodium selenite (15.4 mg/10 kg diet) and their combination on antioxidant enzyme activities along the experimental periods.

	1st week		2nd week		3rd week		4th week	
	SOD	**GPX**	**SOD**	**GPX**	**SOD**	**GPX**	**SOD**	**GPX**
C	29.23±.50^{b}	27.57±.53^{b}	30.08±.52^{b}	28.25±.57^{c}	30.67±.66^{b}	29.31±.44^{b}	31.31±.71^{b}	30.28±.50^{b}
Zn	39.27±.80^{b}	27.76±.69^{b}	41.30±1.19^{a}	28.58±.55^{c}	39.35±.87^{a}	29.15±.53^{b}	38.93±.86^{a}	31.23±.48^{b}
Se	30.98±.04^{a}	35.50±.79^{a}	30.58±.36^{b}	37.18±1.00^{b}	30.40±.33^{b}	40.16±.87^{a}	29.92±.33^{b}	36.17±.87^{a}
Zn + Se	39.88±.89^{a}	37.41±.96^{a}	43.35±1.18^{a}	39.97±1.22^{a}	41.02±.80^{a}	42.28±1.13^{a}	39.75±.79^{a}	35.67±1.0^{a}
LSD	2.42	2.21	2.61	2.57	2.04	2.30	2.05	2.20

Each value represents the mean of 8 birds ± S.E. All data having different letters are differing significantly at p ≤0.05. L S D: Least significant difference. Control (C), zinc (Zn) supplemented ,selenium (Se) supplemented and combination (Zn+Se) groups.SOD = superoxide dismutase, GPX = glutathione peroxidase.

Table 5: Effect of oral supplementation with zinc oxide (2 gm/10 kg diet), sodium selenite (15.4 mg/10 kg diet) and their combination on serum zinc and selenium concentrations (ppm) along the experimental period.

	1st week		2nd week		3rd week		4th week	
	Zn	**Se**	**Zn**	**Se**	**Zn**	**Se**	**Zn**	**Se**
C	1.55±.09b	9.40±.31b	1.54±.09b	9.27±.26b	1.44±.09c	9.09±.25b	1.41±.07b	8.98±.21b
Zn	3.05±.10a	9.19±.26b	3.13±.14a	9.21±.33b	3.11±.27b	9.08±.28b	3.81±.17a	8.90±.22b
Se	1.54±.10b	12.13±.17a	1.42±.08b	13.11±.17a	1.44±.06c	13.75±.18a	1.55±.07b	14.11±.18a
Zn + Se	3.19±.10a	12.35±.21a	3.25±.13a	13.27±.29a	3.60±.166a	13.65±.21a	3.73±.20a	14.47±.39a
LSD	0.29	0.71	0.33	0.79	0.49	0.67	0.41	0.73

Each value represents the mean of 8 birds ± S.E. All data having different letters are differing significantly at p ≤0.05. L S D: Least significant difference. Control (C), zinc (Zn) supplemented ,selenium (Se) supplemented and combination (Zn+Se) groups, Zn = zinc, Se = selenium.

Se is an important component of Se-dependent glutathione peroxidase enzyme, which reduces peroxide and protects cells against the damaging effects of oxidation [45].The analysis of GPx in the present work confirmed the results previously obtained by Payne and Southern [46] who recorded that dietary Se supplementation increased the plasma GPx activity in the broiler chicks. Similarly, Cave et al. [47] suggested that Se supplementation in broiler ration increased the GPx enzyme activity which reduced the oxidative stress. *Khajali et al.* [48] found that organic Se significantly elevated plasma GPx activity at 40 days of bird's age, which can be regarded as an improvement of antioxidant status.

Clinic-biochemical analysis [Figures 3,4] showed no effects of the test supplements on liver and kidney function tests, which may be due to their antioxidative function. The same results were obtained by Yalçinkaya et al. and *Okunlola et al.* [49, 50] .On the other hand, Biswas et al. [37] found a decrease in ALT and AST activities in chicks supplemented with 0.5 mg and 1mg/kg of selenium in their diet. Also, *Gružauskas et al.* [51] found an increase in serum ALT and AST activities in poultry chicks supplemented with 0.5 mg of sodium selenite and others supplemented with 0.15 mg of inorganic selenium and 0.35 mg of organic selenium for 35 days. The variety may be due to the dose differences.

Protein profile (Table 6) in broiler chicks supplemented with selenium and zinc showed an elevation in total proteins, albumin,

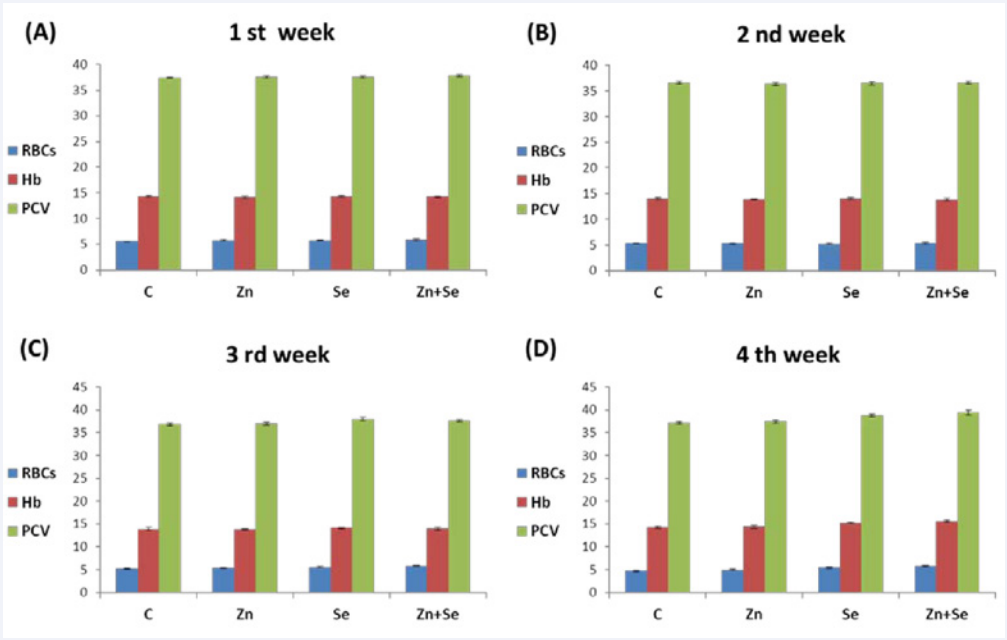

Figure 1 Red blood cell counts(RBCs),hemoglobin concentration (Hb) and packed cell volume (PCV) of control (C), zinc (Zn) supplemented ,selenium (Se) supplemented and combination (Zn+Se) groups. Results were expressed as mean ± S.E. from 8 birds.

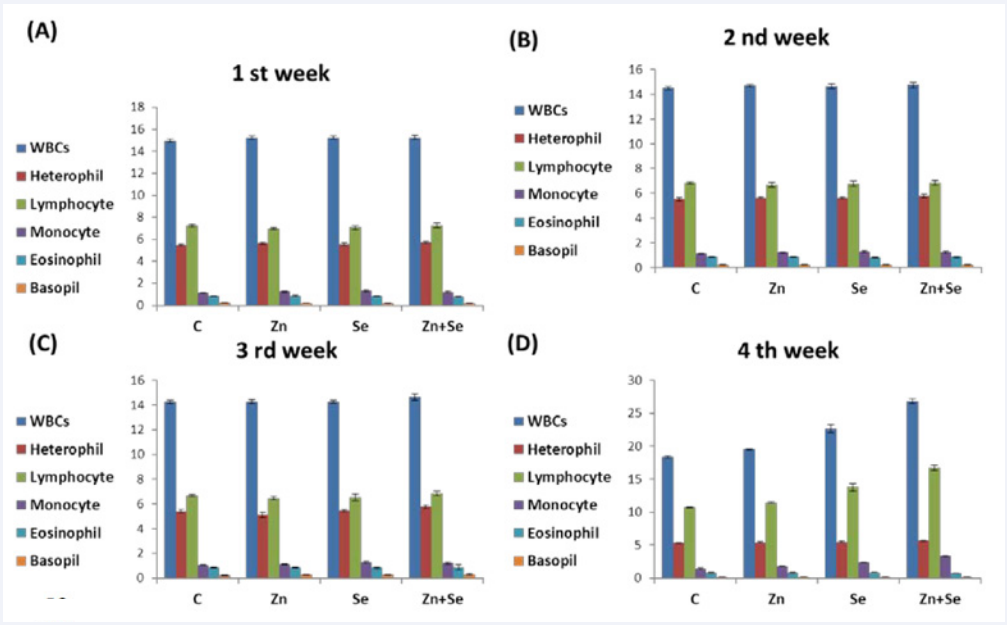

Figure 2 White blood cell counts (WBCs) and differential leukocytic counts of control (C), zinc (Zn) supplemented ,selenium (Se) supplemented and combination (Zn+Se) groups. Results were expressed as mean ± S.E. from 8 birds.

and globulin levels with a decline in albumin/globulin ratio. Similarly, plasma total protein was increased with Zn dietary supplementation in broiler [52] and Zn protein in laying hens [53].These results agreed with the results previously obtained with *Gružauskas et al.* [51] who found an increase of total protein, gamma globulin in poultry chicks supplemented with 0.5 mg of sodium selenite and others supplemented with 0.15 mg of inorganic selenium and 0.35 mg of organic selenium for 35 days.

Related to lipid profile (Table 7), zinc decreased cholesterol, triglycerides and increased HDL-c levels. These results agreed with Uyanmk et al. [54]. On contrary, Lu and Combs [55] reported that inorganic zinc did not affect the serum cholesterol level. Also, selenium caused a decrease in cholesterol and triglycerides and an increase in HDL-c levels. These results agreed with *Gružauskas et al.* [51] who indicated that chicks supplemented 0.15 mg of sodium selenite, 0.35 mg of selenium methionine showed a

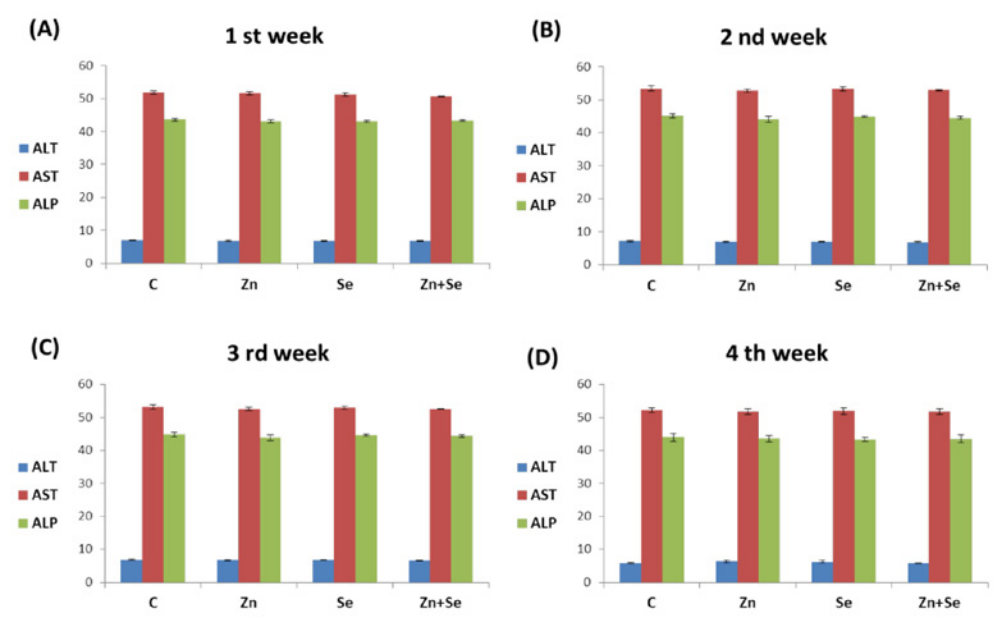

Figure 3 Alanine Transaminase (ALT) , Aspartate Transaminase (AST) and alkaline phosphatase (ALP) of control (C), zinc (Zn) supplemented , selenium (Se) supplemented and combination (Zn+Se) group. Results were expressed as mean ± S.E. from 8 birds.

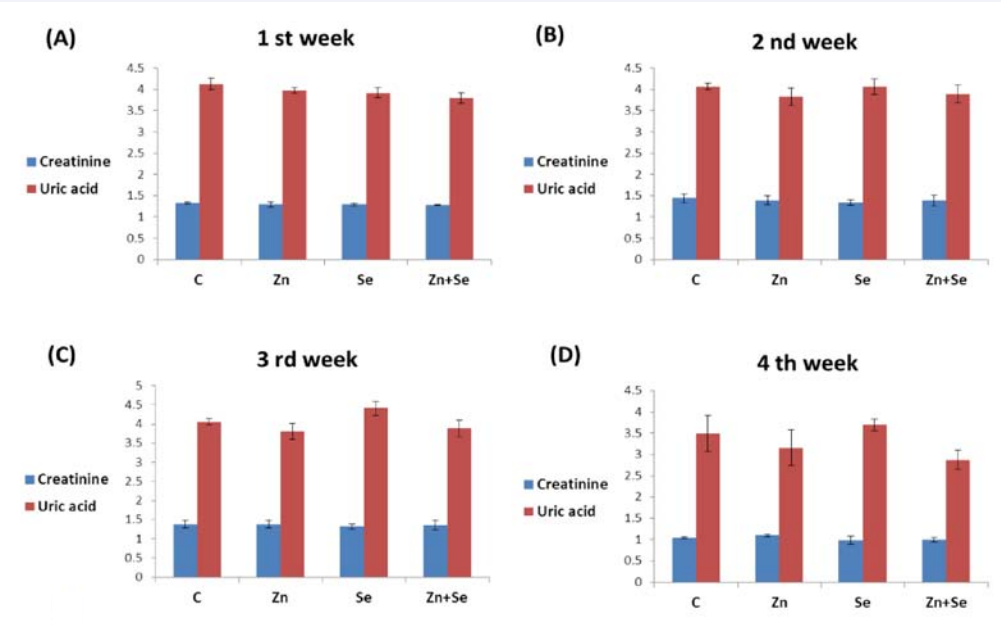

Figure 4 Creatinine and uric acid levels of control (C), zinc (Zn) supplemented , selenium (Se) supplemented and combination (Zn+Se) groups. Results were expressed as mean ± S.E. from 8 birds.

decrease in cholesterol concentration. Moreover, no significant changes in serum glucose levels in chicks supplemented with zinc and selenium were observed. These results disagreed with the results previously obtained by *Al-Daraji* et al. [56] who found that male and female broiler chicks consumed 50, 75, 100 mg pure zinc in their diets for 54, 58, 62 and 66 weeks of age showed significant increase in serum glucose levels in addition to *Gružauskas et al.* [51] who indicated that chicks supplemented 0.15 mg of sodium selenite, 0.35 mg of selenium methionine showed a decrease in glucose concentration.

Addition of zinc to poultry ration increased the hematopoietic system activity [Figure 1] due to an increased serum iron level. This, in turn, could be due to the increase of serum zinc level which stabilizes and regulates the cell membranes functions [57] and protect it from lipid peroxidation [58].Se also increased the erythrocytes counts due to the positive effect on hematopoietic

Table 6: Effect of oral supplementation with zinc oxide (2 gm/10 kg diet), sodium selenite (15.4 mg/10 kg diet) and their combination on total protein, albumin, globulin (g/dl) and albumin/globulin ratio(%) along the experimental periods.

	Total protein	Albumin	Globulin	A/G ratio	Total protein	Albumin	Globulin	A/G ratio
	1st week				2nd week			
C	6.13±.040a	4.56±.11a	1.55±.02a	2.93±.06a	6.63±.07a	4.81±.15a	1.80±.12a	2.84±.363a
Zn	6.18±.040a	4.60±.12a	1.54±.08a	3.06±.16a	6.72±.16a	4.88±.15a	1.79±.15a	2.88±.297a
Se	6.15±.032a	4.57±.09a	1.65±.08a	2.82±.20a	6.78±.12a	4.82±.19a	1.98±.12a	2.52±.226a
Zn + Se	6.25±.066a	4.79±.14a	1.66±.07a	2.92±.18a	6.68±.14a	5.19±.11a	1.98±.06a	2.64±.149a
LSD	0.13	0.34	0.21	0.46	0.38	0.45	0.35	0.78
	3rd week				4th week			
C	6.78±.13b	4.76±.17 a	2.00±.05 a	2.40±.14 a	6.61±.17d	5.04±.08b	1.57±.11d	3.31±.26a
Zn	6.99±.11b	4.81±.13a	1.99±.15 a	2.58±.32 a	7.15±.06c	5.08±.05b	2.06±.03c	2.47±.05b
Se	7.02±.12ab	4.78±.18 a	2.18±.18 a	2.33±.25 a	7.76±.19b	5.36±.17ab	2.40±.03b	2.22±.06bc
Zn + Se	7.33±.04a	5.14±.12 a	2.18±.12 a	2.42±.20 a	8.47±.15a	5.49±.07a	2.98±.12a	1.87±.07c
LSD	0.32	0.45	0.39	0.69	0.55	0.31	0.46	0.41

Each value represents the mean of 8 birds ± S.E. All data having different letters are differing significantly at p ≤0.05. L S D: Least significant difference. Control (C), zinc (Zn) supplemented ,selenium (Se) supplemented and combination (Zn+Se) groups A/G ratio = albumin/globulin ratio.

Table 7: Effect of oral supplementation with zinc oxide (2 gm/10 kg diet), sodium selenite (15.4 mg/10 kg diet) and their combination on lipid profile and glucose level (mg/dl) along the experimental periods.

	Cholesterol	TG	HDL-C	Glucose	Cholesterol	TG	HDL-C	Glucose
	1st week				2nd week			
C	121.2±2.43^{a}	84.5±.51^{a}	63.0±.02^{b}	90.7±.25^{a}	136.6±2.04^{a}	102.6±3.12^{a}	79.1±3.08^{a}	96.0±1.59^{a}
Zn	120.6±.51^{a}	84.4±.45^{a}	63.3±.14^{ab}	90.8±.35^{a}	135.7±3.30^{a}	99.4±2.05^{a}	80.2±2.05^{a}	95.0±1.00^{a}
Se	119.7±1.04^{a}	83.7±.42^{a}	63.4±.13^{a}	90.7±.49^{a}	134.4±2.19^{a}	102.4±2.10^{a}	80.4±3.17^{a}	97.3±1.20^{a}
Zn + Se	118.4±.54^{a}	83.4±.28^{a}	63.1±.20^{b}	90.7±.61^{a}	137.2±1.17^{a}	101.4±1.99^{a}	81.9±1.83^{a}	96.7±1.98^{a}
LSD	3.99	1.24	0.29	1.30	6.63	6.85	7.55	4.33
	3rd week				4th week			
C	138.6±2.23^{a}	104.6±2.1^{a}	79.1±1.55^{b}	107.0±1.8^{a}	138.1±2.56^{a}	121.0±2.28^{a}	86.8±1.47^{c}	120.2±3.51^{a}
Zn	137.6±2.91^{a}	101.4±2.3^{a}	80.2±1.74^{b}	106.0±.53^{a}	116.6±1.41^{b}	106.5±1.69^{b}	88.4±1.36^{bc}	116.8±2.66^{a}
Se	135.5±1.55^{a}	104.4±1.5^{a}	80.4±1.98^{b}	108.3±.99^{a}	103.1±65^{c}	99.6±.77^{c}	91.2±1.41^{b}	118.2±2.11^{a}
Zn + Se	139.0±1.46^{a}	103.2±1.2^{a}	87.8±1.43^{a}	109.3±.98^{a}	91.5±1.77^{d}	88.9±1.78^{d}	95.5±0.93^{a}	120.2±2.30^{a}
LSD	6.15	5.34	4.90	3.50	5.04	4.99	3.80	7.84

Each value represents the mean of 8 birds ± S.E. All data having different letters are differing significantly at p ≤0.05. L S D: Least significant difference. Control (C), zinc (Zn) supplemented ,selenium (Se) supplemented and combination (Zn+Se) groups ,TG = triglyceride, HDL-C = high density lipoproteins.

organs [59].Our results agreed with Biswas et al. [37] and disagreed with *Okunlola et al*. [50] who found that broilers fed on 0.3 mg/kg or 0.5 mg/kg of selenium in their diet showed no significant changes in RBCs, Hb and PCV.

Our leukogram results [Figure 2] are identical to the theory said that selenium has an essential role in immune response optimization, innate and acquired immune systems influence in chicks which might be due to its role in increasing the antioxidant enzymes activities and LP content reduction in the present study. Furthermore, Se enhances the immune responses by reducing the endogenous production of prostaglandin [60]. Similarly, it has been reported that Se markedly stimulates the humoral immune response in broilers up to 30 weeks of age [61].Also; *Zhang* et al. [62] reported that chicks supplemented with selenium in their diets showed improvement in its immunological parameters. Our results partially agreed with Ihsan and Gelawesh [59] who found no significant differences on monocyte, eosinophil and basophil percentages in broilers fed on 0.15,0.3 and 0.45 mg /kg of Se for 42 day, whereas there was a significant decrease in lymphocytes and increases in heterophil percentages and H/L ratio between treatment groups. Also, this result was in agreement with finding of Shlig [63]. Zn also plays an important role in immunomodulation by increasing the counts of thymocytes , peripheral T cells and enhancing the interferon production [7]. Zn increases the T and B lymphocytes, the immune response of bird improves [64].Our results parallel to the study conducted by Bun et al. [65] who showed that dietary supplementation of Zn to broilers infected with *Eimeria tenella* led to an improved immune response. Zn supplementation of broilers increased lymphocyte and proliferation in visceral blood [66]. This finding disagreed with *Donmez* et al. [67] who reported that Zn supplementation did not affect peripheral blood leukocyte counts.

CONCLUSIONS AND APPLICATIONS

1. Zinc oxide and sodium selenite together as chicks feed additives improve the bird's antioxidant status, growth performance, immunity and the hematopoietic system activity.
2. Addition of zinc oxide (2 g /10 kg ration) and sodium selenite (15.4 mg / 10kg ration) for 4 weeks to poultry ration did not induce hepato-nephrotoxicity.

AUTHORS' CONTRIBUTIONS

MMF, HAE planned the study design, MHE, WAMM collected and examined samples, and WAMM drafted and revised the manuscript, read and approved the final manuscript.

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