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Effect of Inclusion Inorganic, Organic or Nano Selenium Forms in Broiler Diets On: 2-Physiological, Immunological and Toxicity Statuses of Broiler Chicks

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Abstract: This study was conducted to evaluate both physiological and immunological efficiency and toxicological effects of different selenium (Se) sources and levels in corn-soybean meal broiler diets. For that, 400 day-old unsexed Arbor Acres broiler chicks were allocated to 10 experimental treatments in a 5 sources x 2 levels factorial design. Five Se sources were tested; (1) sodium selenite (NaSe) as inorganic form; (2) selenomethionine (Se-Yeast) as organic form; (3) Zinc-L-selenomethionine (Zn-Se-Meth) as more recent organic form; (4) powder form of Nano Se form (P-Nano Se) and (5) Liquid form of Nano Se (L-Nano Se). Also two inclusion of Se levels in diets; 0.15 and 0.30 ppm, were examined. The inorganic and organic forms of Se were obtained from commercial suppliers while both powder and liquid forms of Nano Se were prepared immediately before starting feeding phases of the experiment. The prepared 80 nm Se nano-particles were synthesized by chemical reduction method and characterized by Transmission Electron Microscope, X-ray diffraction and spectrophotometry. Three phases (1-10, 11-24 and 25-40 d) of feeding were applied and all birds were kept under similar management conditions. Parameters of blood picture, immunity status, antioxidant status, some plasma constituents and T3 hormone were investigated. Also histological examination of liver samples was carried out at 40 days of age. The obtained results showed significant improvement of some hematological parameters, cellular immunity and antioxidant status either due to using organic or nano forms of Se, or by increasing the inclusion Se level from 0.15 to 0.30 ppm in broiler diets. While humeral immunity against Newcastle Disease Virus and Avian flow Virus (H_5N_1), plasma proteins, activity of liver enzymes and malnodialdhyde (MDA) content in plasma did not affected due to Se sources or levels. Concentration of T3 hormone significantly increased by increasing Se level from 0.15 to 0.30 ppm in the diet. The histological examination of liver showed some severe pathological changes due to increasing Se level from 0.15 to 0.30 ppm for most sources while using 0.15 ppm of Se from inorganic or organic forms of Se showed normal histological structure of liver tissues. The overall experimental results showed although using Zn-Se-Meth as organic form of Se or L-Nano Se as nano form of Se or increasing the supplemental Se to 0.30 ppm in broiler diets or its equivalent in drinking water is more effective to get better, physiological, immunological and antioxidant status of broiler chicks. Inclusion Se-Yeast as organic form of Se in broiler diets at level 0.15 ppm was more save to liver tissues and kidney function. Further studies about the safety of using nano form of selenium as feed additives are needed.

Key words: Broiler, inorganic selenium, organic selenium, nano selenium, physiology, immunity, toxicity

INTRODUCTION

Selenium is an essential trace element that has a large number of biological functions in poultry. Selenium is sometimes supplemented in broiler diets in its inorganic form (sodium selenite). However, this salt is very toxic and needs to be more soluble in its ionic form in order to increase its absorption in gastrointestinal tract. In addition, the electric charges of this ionic form may interact with other diet components (minerals, proteins and carbohydrates), rendering them partially unavailable to animals (Saad *et al*., 2013). This has stimulated research on the use of organic selenium improve its bioavailability.

Many researches study the various effects of using selenomethionine as organic selenium source in broiler or layer diets and most of them reported positive effects (El-Sebai, 2000; Mahmoud and Edens, 2003; El-Sheikh *et al*., 2006). Selenomethionine is the most appropriate form of Se for use in animal nutritional supplements because of their excellent bioavailability and lower toxicity among various forms of Se (Utterback *et al*., 2005). Also there is more recent organic selenium source which is Zn-L-selenomethionine (Zn-Se-Meth). As described by producers, it may be more effective organic selenium source. Zinc-L-selenomethionine is designed to be highly soluble and increase bioavailability of Se (Ward, 2003). Until now there are no available references about evaluation of Zn-Se-Meth in broiler feeding.

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With the recent development of nanotechnology, nano-selenium (nano-Se) has attracted widespread attention because nanometer particulates exhibit novel characteristics such as a large surface area, high surface activity, high catalytic efficiency, strong adsorbing ability and low toxicity (Wang *et al*., 2007; Zhang *et al*., 2008). It has been reported that nano-Se possesses comparable efficiency to selenite and Se-methylselenocysteine in upregulating selenoenzymes but with dramatically decreased toxicity (Zhang *et al*., 2008). The different physiological effects of nano-Se and sodium selenite were probably related to the different absorption process and metabolic pathways (Mohapatra *et al*., 2014). It has been reported that nanoparticle show new characteristics of transport, uptake and exhibit higher absorption efficiencies (Liao *et al*., 2010). They suggested that the superior performance of nanoparticles may be attributed to their smaller particle size and larger surface area, increased mucosal permeability, improved intestinal absorption and tissue depositions. The transport efficiencies of selenomethionine and nano-Se were higher than that of sodium selenite ($p<0.05$). The highest uptake efficiency ($p<0.05$) was observed in cells treated with nano-Se and significant difference was also observed between the cells incubated with sodium selenite and selenomethionine (Wang and Fu, 2012).

Selenium supplementation to the broiler chickens diet caused a significant increase in RBC's counts and other hematological parameters (El-Sebai, 2000; Abaza, 2002). Biswas *et al*. (2011) and Ihsan and Qader (2012) reported that total erythrocyte count (TEC), packed cell volume (PCV) and hemoglobin (Hb) content significantly increased in the treated groups with Se as compared to the control group. They added that increasing level of TEC, Hb content and PCV might be due to the effects on hemopoietic organs. Selenium is involved in antibody production and plays a major role against diseases (Kidd, 2004). Ihsan and Qader (2012) reported that selenium leads to improve the immunity in broiler by decreasing of heterophils to lymphocytes ratio at 6 weeks of age. Selenomethionine improves chicken immune status by increasing ability of immuno-competent cells to respond to antigen stimuli (Leng *et al*., 2003).

Glutathione peroxidase (GSH-Px) has antioxidative action and contributes to the oxidative defense by catalyzing the reduction of hydrogen peroxide and lipid peroxides to less harmful hydroxides (Arthur, 2000). The activity level of this enzyme in the liver or plasma is indicative of the Se supply to the organism moreover antioxidant protection levels are affected by dietary Se status (Wang and Xu, 2008; Wang, 2009). Heat stress, a potent inducer of oxidative stress, can be partially ameliorated by feeding organic selenium. This observation attribute to an enhanced antioxidant system with organic selenium (Mahmoud and Edens, 2003).

Nano-Se was shown to be similar in increasing the activities of GSH-Px and thioredoxin reductase, although nano-Se had a much lower toxicity compared with selenomethionine (Wang *et al*., 2007). Significant quadratic effect of nano-Se was observed on glutathione peroxidase activity, free radical inhibition, contents of IgM, glutathione and malondialdehyde in serum, on glutathione peroxidase activity, free radical inhibition in liver and on glutathione peroxidase activity in muscle, with birds fed 0.30 ppm of nano-Se exhibiting the best effect and birds fed 2.0 ppm of nano-Se showing the worst effect on these parameters (Cai *et al*., 2012). Supplementing the diet with nano- Se also significantly increased the GSH-Px activity (Zhou and Wang, 2011).

The TAOC reflects the total antioxidant capacity of the body. Low TAOC could be an indication of oxidative stress or higher susceptibility to oxidative damage (Ahmad *et al*., 2012). Compared with the control at 28 day, the TAOC in plasma significantly increased at the range of 0.3 to 1.2 mg/kg nano-Se (Fu-xiang *et al*., 2008). The objective of this study, evaluated the effects of using different forms of selenium at levels of 0.15 or 0.30 ppm in broiler diets on physiological, immunological and toxicity statuses of broiler chicks.

MATERIALS AND METHODS

This study has done in Animal Production Research Institute (APRI) and with collaboration with Nanotechnology and Advanced Materials Central Lab (NAMCL), Agricultural Research Center (ARC), Giza, Egypt.

Nano selenium synthesis and characterization: Selenium nano-particles (SeNPs) were prepared according to Zhang *et al*. (2004) as described in the first part of this study. In brief, 100 ml of 1 mM Sodium Selenite heated under stirring, then 2.5 mL of 1% Ascorbic Acid was added drop wise until the color change to the characteristic yellowish orange and left to cool with stirring for 30 min. The prepared Se colloid was undergo characterization by Transmission Electron Microscope (HR-TEM, Tecnia, G20, 200 Kv, FEI, Netherland), X-ray diffraction (X'pert Pro, Pan Alytical, Netherland), Particle size analyzer (ZS, Malvern, UK) and spectrophotometry (Cary 5000, Varian, UK). All used chemicals were obtained from Sigma-Aldrich and used as purchased without any modification. Synthesis and characterization was done at NAMCL. The synthesized SeNPs were prepared in powder and liquid forms (P-Nano Se and L-Nano Se) as described in the first part of this study. The P-Nano Se was added to the experimental diets at levels 0.15 or 0.30 ppm and L-Nano Se solution was added to drinking water on equivalent levels of that added to diet.

Experimental procedures: Four hundred one day old unsexed Arbor Acres broiler chicks were obtained from

a commercial hatchery individually weighed and assigned to 10 dietary treatments (4 replicates/treatment of 10 chicks each) to study the effect of different sources and levels of supplemental Se. Five Se sources were tested; (1) sodium selenite, NaSe; (2) selenomethionine, Se-Yeast; (3) Zinc-L-selenomethionine, Zn-Se-Meth; (4) powder form of SeNP, P-Nano Se and (5) Liquid form of SeNP, L-Nano Se and two levels of Se supplementation; 0.15 and 0.30 ppm in broiler diets, were examined. The liquid form of Nano Se was added in drinking water daily at equivalent doses using feed consumption of chicks in control group for the previous day as reference. Chicks fed on corn-soybean meal basal diets which meet the strain requirements excluding Se during starting (1-10 d), growing (11-24 d) and finishing (25-40 d) periods (Table 1). The experimental design is shown in Table 2. All birds were kept under similar management conditions. The environmental temperature and humidity surrounding birds were recorded daily during the experimental period. Live body weights and feed consumption of chicks were recorded at 10, 24 and 40 days of age, then live body weight gains and feed conversion ratios were calculated.

Table 1: Composition and calculated analysis of basal diet

Composition (per 100 kg)	Starter (1-10 day)	Grower (11-24 day)	Finisher (25-40 day)
Yellow Corn	52.28	59.05	63.19
Soybean meal (44%CP)	34.00	26.70	22.5
Corn gluten (60% CP)	6.00	7.00	6.30
Soy bean oil	3.00	3.00	4.00
Di-Calcium phosphate	1.84	1.67	1.59
Lime stone	1.43	1.20	1.10
L-Lysine HCl	0.32	0.31	0.28
Dl-Methionine	0.26	0.20	0.17
Sodium chloride	0.24	0.24	0.24
Sodium bicarbonate	0.23	0.23	0.23
Vitamins Premix *	0.10	0.10	0.10
Minerals Premix**	0.30	0.30	0.30
Total	100.00	100.00	100.00
Calculated analysis (%)			
Crude protein	23.17	21.25	19.04
Metabolizable energy (Kcal/kg)	3100	3110	3207
Ether extract	5.63	5.08	6.88
Crude fiber	3.80	3.45	3.22
Calcium	1.04	0.90	0.84
Av. Phosphorus	0.50	0.45	0.43
Lysine	1.44	1.24	1.09
Methionine	0.68	0.60	0.54
Methionine+cystine	1.06	0.95	0.86
Sodium	0.15	0.16	0.17

*Supplied per kg of diet: Vit. A, 11000 IU; Vit. D3, 5000 IU; Vit. E, 50 mg; Vit K3, 3 mg; Vit. B1, 2 mg; Vit. B2 6 mg; B6 3 mg; B12, 14 mcg; Nicotinic acid 60mg; Folic acid 1.75 mg, Pantothenic acid 13mg; and Biotin 120 mcg

**Supplied per kg of diet: Choline 600 mg; Copper 16 mg; Iron 40 mg; Manganese. 120 mg; Zinc 100 mg and Iodine 1.25 mg

Table 2: Experimental design

	Supplementation of Se sources and levels to basal diet in ppm (1-40 d of age)									
	1	2	3	4	5	6	7	8	9	10
NaSe	0.15	0.30								
Se-Yeast[1]			0.15	0.30						
Zn-Se-Meth[2]					0.15	0.30				
P-Nano Se[3]							0.15	0.30		
L-Nano Se[4]									0.15	0.30

1: Sel-plex®; 2: Availa-Se®; 3: Prepared in NAMCL and added in diet; 4: Prepared in NAMCL and added to drinking water

Physiological traits

Blood sampling and analysis: At the end of the experimental period (40 d of age), 4 birds/treatment (bird/replicate), around the average body weight, were chosen and three blood samples were collected from each bird in heparinized tubes (2 complete blood sample and 1 plasma sample/bird). The heparinized plasma samples kept at -20°C until the time of chemical analyses of Total protein (TP), Albumin (Alb), Triiodothyronine hormone (T3), Creatinine, Alkaline Phosphatase activity (Alk), Aspartate transaminase (AST), Alanine transaminase (ALT), Total antioxidant capacity (TAOC) and Malondialdehyde (MDA) by colorimetric methods. Plasma globulin (Glo) was calculated by difference between TP and Alb also ratio between Alb and Glo (A/G) was calculated by dividing. While the first complete blood sample was used to determine blood hematological parameters according to Clark *et al*. (2009) and the second sample kept at 4°C for then sent directly to the blood analysis lab in APRI to determine activity of glutathione peroxidase activity (GSH-Px) in red blood cells. All blood examinations were done by using analytical kits produced by Biodiagnostic Company.

Immunological response: At 40 d of age one whole blood sample/bird (4 samples/treatment) were used to examined the immune response to Newcastle Disease Virus (NDV) and Avian flow Virus (H_5N_1) by measuring titer against these viruses using preventing from hemangontinasion method and manual of diagnostic tests and vaccines for terrestrial animals, respectively. These examinations were carried out in Reference Lab for Veterinary Quality Control on Poultry Production, Egypt.

Liver tissues examinations: At 40 d of age, 40 birds (4 birds per treatment which were around the average body weight) were slaughtered and Liver of each slaughtered chicks were removed carefully, weighed and fixed in 10% buffered neutral formalin, dehydrated in ascending grades of alcohol, then cleared with xylene and embedded in paraffin, sectioned at 5 µm thickness and stained by H and E stain, Masson's trichrome stain, periodic acid Schiff's (PAS) and Prussian blue stains and then examined microscopically (Bancroft and Stevens, 1990).

Statistical analysis: Data of experimental treatments were analyzed by using two way analysis of variance to

detect the effect of selenium source and supplemental level. Also data of all experimental treatments, were analyzed by using one way analysis of variance to detect the best treatment between them. Variables showed significant differences at F-test ($p \leq 0.05$) were compared to each other's using Duncan's Multiple Range Test (Duncan, 1955). The statistical procedures were computed using SAS (1999).

RESULTS

During the experimental period (1 to 40 d of age) environmental temperature and relative humidity% surrounding chicks were recorded daily and there values were ranged between 36-41°C and 30-55%, respectively.

Characterization of selenium nanoparticles: The optical properties of the synthesized SeNPs have been characterized by their Plasmon absorbance band at 265 nm with Gaussian distribution indicating formation of spherical SeNPs with no aggregation, uniformity and excellent dispersion of colloidal Se nano-particles (Fig. 1). The high resolution-TEM (HR-TEM) image of SeNPs shows monodisperse spherical shape with average size 80 nm, (Fig. 2). XRD measurement were employed to investigate the phase and structure of the synthesized sample (Fig. 3) shows the XRD pattern of the as prepared SeNPs suggesting that the sample in nano size matched well with the data from the JCPDS card (1-086-2246).

Hematological examination: Data in Table 3 showed hematological parameters including total erythrocytes count (TEC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV = Ht x 10/TEC), mean corpuscular hemoglobin (MCH = Hb x 10/TEC), mean corpuscular hemoglobin concentration (MCHC = Hb x 100/Ht), heterophils% (H), lymphocytes% (L) and H/L ratio. The presented values showed adverse effect of adding NaSe in broiler diets on values of TEC, Hb and Ht of chicks at 40 days of age compared with other organic and nano Se sources, while increasing level of supplementation from 0.15 to 0.30 ppm did not change values of hematological parameters significantly. Neither supplemental Se sources nor levels change MCV "the average volume of red blood cell" or MCH and this indicate that neither a microcytic anemia (MCV below normal range), nor macrocytic anemia (MCV above normal range) observed among treatments. On the same manner (MCH) "the average mass of hemoglobin per red blood cell in a sample of blood" didn't significantly differ.

Cellular and humeral immunity: The obtained results showed significant increase of L% and significant decrease of both H% and H/L ratio by used organic or nano forms of Se compared with inorganic form (Table 3). In addition, the same trend was found by increasing supplemental Se level from 0.15 to 0.30 ppm. Samples of L-Nano Se-0.30 and Zn-Se-Meth-0.30 chicks showed the lowest values of H% and H/L while those of chicks in Se-Yeast-0.15, P-Nano Se-0.30 and Zn-Se-Meth-0.30 showed the highest values of L%. On the other side, results of humeral immunity showed that antibodies titer against NDV and H_5N_1 didn't affected by Supplemental Se sources, levels or their interactions.

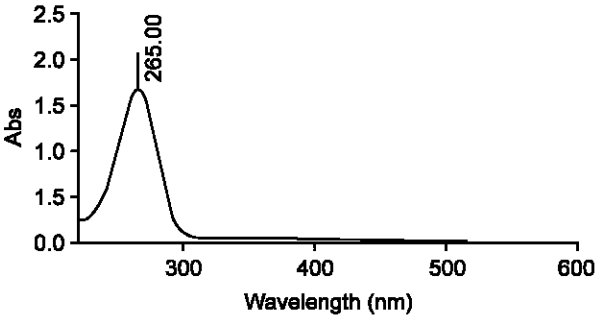


Fig. 1: Shows the UV-Vis spectrum of SeNPs with absorption peak at 265 nm

Plasma constituents: As shown in Table 4, plasma total proteins, albumin, globulins and albumin/globulins ratio didn't significantly affected due to experimental main factors or interactions and agreement with the results of antibodies titer, where antibodies are fraction of globulin. The same trend recorded for activity of liver enzymes including ALT, AST and ALK. While the determined values of Creatinine showed significant effect of Se source on kidney function. Although, adding Zn-Se-Meth, P-Nano Se, or L-Nano Se in broiler diets resulted in increase plasma Creatinine level compared with values in Se-yeast chicks which resulted in the lowest Creatinine values. Increasing supplemental Se level from 0.15 to 0.30 ppm in broiler diets could not cause any significant difference in plasma Creatinine level. Chicks of Se-Yeast-0.15 and Se-Yeast-0.30 groups recorded lower significant values of Creatinine compared with that of Zn-Se-Meth-0.30, P-Nano Se-0.30 and L-Nano Se-0.30 groups.

Antioxidant status: The determined values of antioxidant status indicators showed significant increase of GSH-Px activity by inclusion of Zn-Se-Meth or L-Nano Se in broiler diets (35.5 and 29%, respectively) compared to NaSe determined value. The same trend recorded by increasing Se supplementation level from 0.15 to 0.30 ppm (16.4% increases). Similarly, determined values of TAOC showed 39.5 and 33.2% increase by adding Zn-Se-Meth or L-Nano Se to broiler diets comparing with NaSe group and 19.5% increase of TAOC by elevating Se supplementation level from 0.15

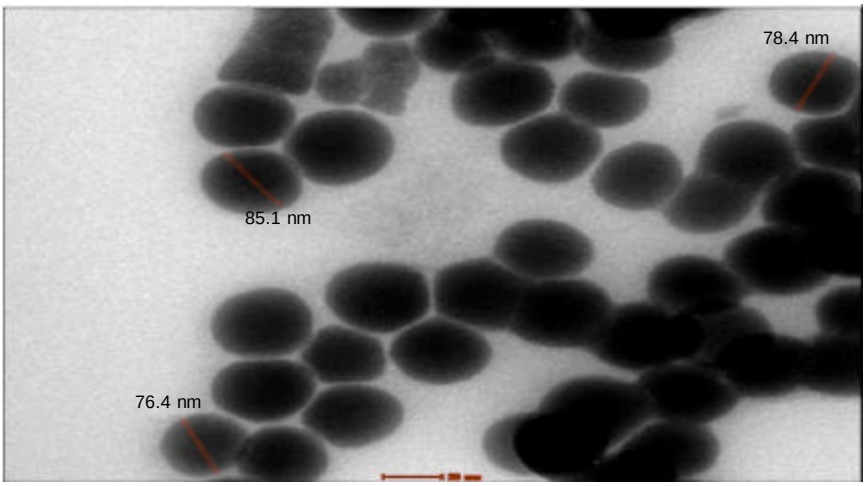


Fig. 2: HR-TEM image of SeNPs with average size 80 nm

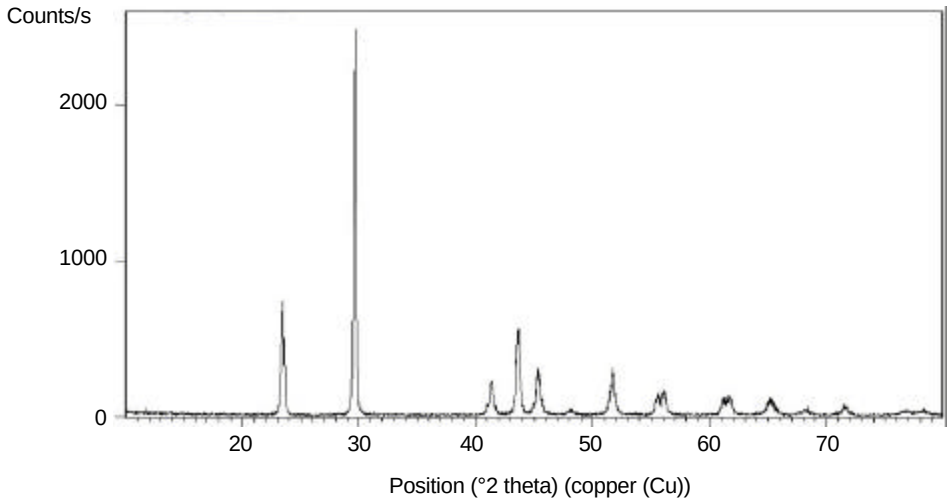


Fig. 3: XRD pattern of SeNPs

to 0.30 ppm. While values of MDA did not show any significant differences due to main factors or their interactions. Among experimental treatments the highest antioxidant status in blood samples recorded for Zn-Se-Meth-0.30 and L-Nano Se-0.30.

The determined values of thyroid T3 hormone showed increased values due to increasing supplemental Se levels from 0.15 to 0.30 ppm (from 153 to 174 ng/L, 13.73%) while using different sources of Se in broiler diets could not cause any significant difference in T3 values at 40 days of age.

Histological examination of liver tissues: Figure 4 display some pictures of the histological examination of liver samples of 40 days broiler chicks from all experimental treatments. While the histological examination of liver tissue of chicks in NaSe-0.15 group showed normal histological structure, the presented picture of NaSe-0.30 showed pathological changes as focal large area of necrosis replaced and displaced with large area of inflammatory cells mainly macrophage and inflammatory cells. Also inflammatory cells were noticed around the blood vessels. Among slides of different groups there was no histological change of liver tissue by feeding chicks on the lower supplemental Se level from Zn-Se-Meth or Se-Yeast sources, while increasing Se level to 0.30 ppm in broiler diets from that sources caused some changes like thickening of the wall of blood vessels, focal inflammatory cells aggregation (Se-Yeast-0.30), congestion of central veins and sinusoids and inflammatory cells aggregations between the hepatocytes (Zn-Se-Meth-0.30) were only changes

Table 3: Effect of different Se sources and levels on Hematological parameters and Immunity of broiler chicks at 40 days of age

	Hematological parameters						Cellular immunity			Humeral immunity	
Treatments	TEC ($X10^6/mm^3$)	Hb (g/dl)	Ht (%)	MCV	MCH	MCHC (g/dl)	H (%)	L (%)	H/L	NDV	H_5N_1
Effect of selenium source											
NaSe	3.96^b	13.56^b	35.21^b	89.13	34.33	38.50	30.60^a	63.53^b	0.482^a	8.73	7.83
Se-Yeast	4.23^a	13.92^a	36.66^a	86.75	32.93	37.96	28.00^b	66.33^a	0.422^b	8.90	7.86
Zn-Se-Meth	4.20^a	14.05^a	36.71^a	87.63	33.51	38.25	27.91^b	66.10^a	0.424^b	8.92	7.97
P-Nano Se	4.17^a	14.01^a	36.25^a	87.23	33.73	38.65	28.26^b	65.78^a	0.430^b	8.85	7.93
L-Nano Se	4.13^a	13.90^a	36.11^a	87.43	33.65	38.46	28.45^b	65.13^a	0.437^b	8.83	7.81
SEM	±0.027	±0.042	±0.138	±0.350	±0.158	±0.095	±0.335	±0.298	±0.007	±0.036	±0.049
Probability	0.01	0.001	0.001	N.S	N.S	N.S	0.02	0.01	0.01	N.S	N.S
Effect of selenium level (ppm)											
0.15	4.10	13.88	36.01	87.90	33.91	38.56	29.33^a	64.78^b	0.453^a	8.80	7.79
0.30	4.17	13.89	36.37	87.36	33.35	38.16	27.96^b	65.96^a	0.424^b	8.89	7.97
SEM	±0.027	±0.042	±0.138	±0.350	±0.158	±0.095	±0.335	±0.298	±0.007	±0.036	±0.049
Probability	N.S	N.S	N.S	N.S	N.S	N.S	0.01	0.01	0.01	N.S	N.S
Interaction between selenium source and level											
NaSe-0.15	3.96^c	13.66^{bc}	35.16^c	89.03	34.56	38.83	31.10^a	63.36c	0.490^a	8.66	7.66
NaSe-0.30	3.97^c	13.46^c	35.26^c	89.23	34.10	38.16	30.10^{ab}	63.70c	0.473^{ab}	8.80	8.00
Se-Yeast -0.15	4.26^{ab}	13.90^{ab}	36.60^{ab}	86.13	32.73	38.00	27.60^{bc}	66.40^a	0.416^{bcd}	8.86	7.80
Se-Yeast -0.30	4.20^{ab}	13.94^{ab}	36.73^{ab}	87.36	33.13	37.93	28.40^{abc}	66.26^{ab}	0.429^{bcd}	8.93	7.93
Zn-Se-Meth-0.15	4.10^{abc}	13.96^{ab}	36.43^{ab}	88.93	34.10	38.33	29.00^{abc}	65.23^{abc}	0.446^{abcd}	8.83	7.83
Zn-Se-Meth-0.30	4.30^a	14.13^a	37.00^a	86.33	32.93	38.17	26.83c	66.96^a	0.400^d	9.00	8.10
P-Nano Se-0.15	4.06^{bc}	13.97^{ab}	36.03^{abc}	88.66	34.40	38.76	29.06^{abc}	64.90^{abc}	0.448^{abcd}	8.87	7.94
P-Nano Se-0.30	4.27^{ab}	14.07^a	36.46^{ab}	85.80	33.06	38.53	27.46^{bc}	66.66^a	0.412^{cd}	8.83	7.93
L-Nano Se-0.15	4.13^{abc}	13.93^{ab}	35.83^{bc}	86.76	33.76	38.90	29.90^{ab}	64.03^{bc}	0.467^{abc}	8.76	7.73
L-Nano Se-0.30	4.13^{abc}	13.86^{ab}	36.40^{ab}	88.10	33.53	38.03	27.00c	66.23^{ab}	0.408^d	8.90	7.90
SEM	±0.027	±0.042	±0.138	±0.350	±0.158	±0.095	±0.335	±0.298	±0.007	±0.036	±0.049
Probability	0.01	0.01	0.01	N.S	N.S	N.S	0.02	0.01	0.01	N.S	N.S

a,b: Means in the same column with different superscripts, differ significantly ($p<0.05$); NS: Non significant ($p>0.05$); SEM: Standard Error of Means

observed in the liver. On the other side using nano-Se sources in broiler diets or in drinking water resulted in clear pathological changes as aggregation of inflammatory cells and focal areas of necrosis with mononuclear inflammatory cells aggregation around the blood vessels in the portal areas and hydropic degeneration of cells.

DISCUSSION

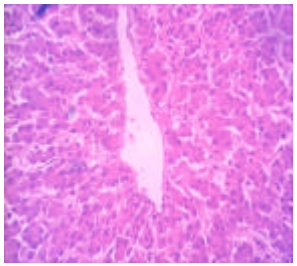
The obtained results of hematological examination showed significantly higher TEC, PCV and Hb values by adding organic or nano forms of Se compared to inorganic selenium. These results reported previously by Hanafy *et al.* (2009) and El-Sheikh *et al.* (2010) who reported that organic selenium supplementation at 0.2 and 0.3 ppm significantly increased the concentrations of Hb. Mohapatra *et al.* (2014) reported that nano-Se appeared to be more effective ($p<0.05$) in increasing different hematological parameters than that of inorganic sodium selenite at level of 0.3 ppm. Selenium has antioxidant effect on the red blood cell membrane, it prevents the degradation of the mature erythrocytes. Because of the intensification of the erythropoiesis, the red blood cell count and the hemoglobin value increase (Raduta *et al.*, 2011). So Se improved markedly hematological parameters (Huang *et al.*, 1999). Increasing hematological parameters slightly than the normal range might be due to higher availability of organic and nano forms of Se for livestock and higher antioxidant protection compared with inorganic selenium (Mahmoud and Edens, 2003).

Results of cellular immunity Showed that Lymphocytes significantly increased, while heterophilous and H/L ratio significantly decreased by organic selenium treatments. These results were in agreement with (Ihsan and Qader, 2012; Shlig, 2009) who stated that there were significant differences ($p<0.05$) on lymphocytes, heterophilus percentages and H/L ratio between Se treatment groups. This may be due to organic selenium improve the immunomodulating properties than inorganic selenium (Surai, 2006). Nano selenium supplementation significantly increased lymphocytes while heterophilus and H/L ratio significantly decreased. The results in agreement with Fu-xiang *et al.* (2008) who reported that when nano-Se supplemented lymphocytes significantly increased compared with control (zero nano-Se supplementation). This may be due to nano-Se increased cellular immunity (Mohapatra *et al.*, 2014). Concerning results of humeral immunity, the antibodies titer against NDV and H_5N_1 didn't affected by Se treatments and was in agreement with results of Saad *et al.* (2013) who reported no differences in antibodies titer against NDV between organic and inorganic selenium. The same trend was reported by Mohapatra *et al.* (2014) who showed no differences in antibodies titers between sodium selenite (0.3 mg/kg diet) and nano-Se (0.15, 0.3 and 0.60 mg/kg diet). In contrast Wang *et al.* (2008) recorded increased antibodies titer against NDV by adding nano-Se at levels between 0.15 and 1.2 mg/kg diet compared with unsupplemented control group. The Conflicting results

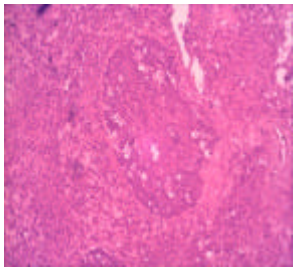
Table 4: Effect of different Se sources and levels on functions of liver and kidney and antioxidant status of broiler chicks at 40 days of age

	Plasma proteins				Kidneys and liver functions				Antioxidant status			
Treatments	TP mg/L	Alb mg/L	Glo mg/L	A/G	Creatinine (mg/dl)	ALT (U/L)	AST (U/L)	Alk (U/L)	GSH-Px (mU/ml)	TAOC (mmol/l)	MDA (nmol/ml)	T3 (ng/dl)
Effect of selenium source												
NaSe	5.48	3.20	2.29	1.431	4.75^{ab}	89.45	156.52	147.16	0.403^{b}	0.430^{b}	2.713	163
Se-Yeast	5.60	3.18	2.42	1.356	4.04^{b}	88.16	156.00	147.73	0.468^{ab}	0.508^{ab}	2.608	160
Zn-Se-Meth	5.94	3.45	2.50	1.386	5.50^{a}	90.15	156.15	147.30	0.546^{a}	0.600^{a}	2.506	170
P-Nano Se	5.86	3.33	2.53	1.353	5.69^{a}	90.80	159.43	147.26	0.500^{ab}	0.533^{ab}	2.500	165
L-Nano Se	5.53	3.15	2.37	1.400	5.45^{a}	90.18	157.13	147.35	0.520^{a}	0.573^{a}	2.416	162
SEM	±0.103	±0.082	±0.073	±0.059	±0.166	±0.345	±0.898	±0.168	±0.017	±0.020	±0.049	±3.90
Probability	N.S	N.S	N.S	N.S	0.01	N.S	N.S	N.S	0.0403	0.036	N.S	N.S
Effect of selenium level (ppm)												
0.15	5.44	3.16	2.27	1.413	4.85	88.9733	155.78	147.10	0.450^{b}	0.482^{b}	2.630	153^{b}
0.3	5.92	3.35	2.57	1.358	5.31	90.53	158.31	147.62	0.524^{a}	0.576^{a}	2.468	174^{a}
SEM	±0.103	±0.082	±0.073	±0.059	±0.166	±0.345	±0.898	±0.168	±0.017	±0.020	±0.049	±3.90
Probability	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	0.0162	0.01	N.S	0.012
Interaction between selenium source and level												
NaSe-0.15	5.37	3.14	2.23	1.443	4.56^{bc}	88.63	155.67	147.03	0.373^{d}	0.396^{c}	2.790	150
NaSe-0.30	5.60	3.24	2.35	1.420	4.92^{abc}	90.27	157.36	147.30	0.433^{bcd}	0.463^{bc}	2.636	175
Se-Yeast -0.15	5.47	3.19	2.28	1.410	3.95^{c}	87.03	155.33	147.10	0.400^{cd}	0.430^{bc}	2.716	142
Se-Yeast -0.30	5.74	3.17	2.57	1.303	4.12^{c}	89.30	156.67	148.36	0.536^{abc}	0.586^{ab}	2.500	178
Zn-Se-Meth-0.15	5.56	3.24	2.32	1.400	5.25^{abc}	89.26	155.53	147.17	0.500^{abcd}	0.550^{abc}	2.540	161
Zn-Se-Meth-0.30	6.32	3.65	2.66	1.373	5.75^{ab}	91.03	156.76	147.43	0.593^{a}	0.653^{a}	2.473	179
P-Nano Se-0.15	5.46	3.16	2.30	1.377	5.33^{abc}	90.13	156.57	147.22	0.520^{abcd}	0.536^{abc}	2.523	160
P-Nano Se-0.30	6.27	3.51	2.76	1.330	6.05^{a}	91.467	162.30	147.32	0.480^{abcd}	0.530^{abc}	2.476	171
L-Nano Se-0.15	5.35	3.10	2.26	1.436	5.16^{abc}	89.80	155.80	147.00	0.460^{abcd}	0.500^{abc}	2.580	153
L-Nano Se-0.30	5.69	3.20	2.49	1.363	5.73^{ab}	90.57	158.46	147.70	0.580^{ab}	0.646^{a}	2.253	170
SEM	±0.103	±0.082	±0.073	±0.059	±0.166	±0.345	±0.898	±0.168	±0.017	±0.020	±0.049	±3.90
Probability	N.S	N.S	N.S	N.S	0.03	N.S	N.S	N.S	0.0319	0.033	N.S	N.S

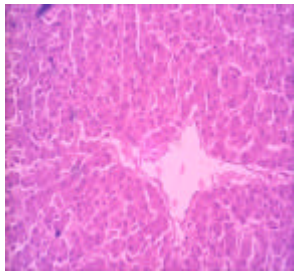
a,b: Means in the same column with different superscripts, differ significantly ($p<0.05$); NS: Non significant ($p>0.05$); SEM: Standard error of means



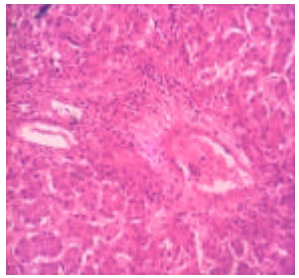
NaSe-0.15: Liver of chicks in control group showing the normal histological structure of the Central Vein (CV) and surrounding Hepatocytes (H) [H&E x 40].



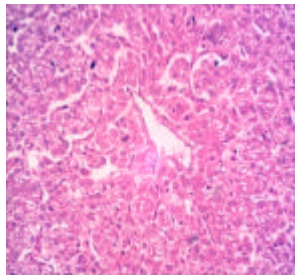
NaSe-0.30: Liver of chicks in this group showing severe pathological changes as necrosis and large area of inflammatory cells. The hepatocytes shows vacuolar degeneration [H&E x 40].



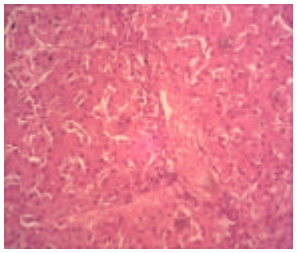
Se-Yeast-0.15: Liver of chicks in this group showing the normal histological structure of the Central Vein and surrounding Hepatocytes [H&E x 40].



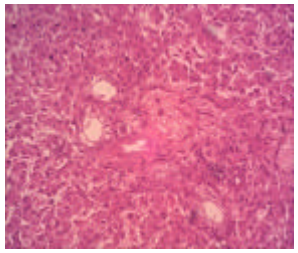
Se-Yeast-0.30: Liver of chicks in this group showing thickening of the wall of blood vessels and focal inflammatory cells aggregation [H&E x 40].



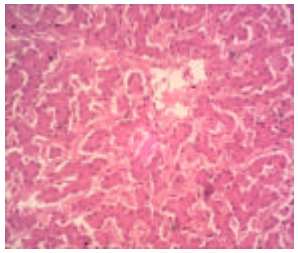
Zn-Se-Meth-0.15: Liver of chicks in this group showing normal histological appearance [H&E x 40].



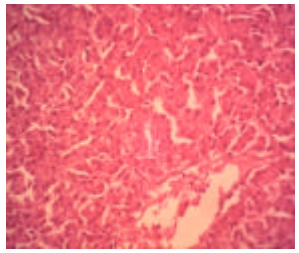
Zn-Se-Meth-0.30: Liver of chicks in this group showing congestion of central veins and sinusoids. Inflammatory cells aggregations were detected in between the hepatocytes [H&E x 40].



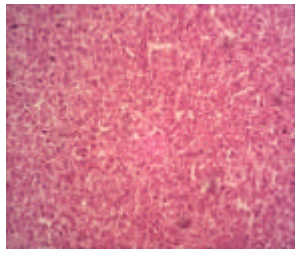
P-Nano Se-0.15: Liver of chicks in this group showing Slight hydropic degeneration in some hepatocytes. There were focal aggregations of plasma cells around the blood vessels in the portal areas [H&E x 40].



P-Nano Se-0.30: Liver of chicks in this group showing focal aggregation of inflammatory cells [H&E x 40].



L-Nano Se-0.15: Liver of chicks in this group showing focal areas of necrosis with mononuclear inflammatory cells aggregation [H&E x 40].



L-Nano Se-0.30: Liver of chicks in this group showing slight hydropic degeneration [H&E x 40].

Fig. 4: The histological examination of chicks liver tissues fed on different Se sources and levels from 1 to 40 days of age

of cellular and humeral immunity in agreement with Baowei *et al.* (2011) who reported that Se supplementation enhanced the organ and cellular immunity, but did not alter the humeral immunity. This may be due to Se has been shown to stimulate the transformation of T lymphocytes into cytotoxic cells (Kiremidjian-Schumacher *et al.*, 1994; Leng *et al.*, 2003). Cytotoxic cells are T lymphocyte that kills cancer cells, cells that are infected (particularly with viruses), or cells that are damaged in other ways. In previous studies, Yang *et al.* (2012) and Mohapatra *et al.* (2014) found that liver enzymes didn't affected by adding different forms of Se (inorganic, organic or nano) at levels up to 0.3 mg Se/kg diet.

Our results of antioxidant status showed significant increase of GSH-Px activity and TAOC values due to adding Zn-Se-Meth or L-Nano Se compared with inorganic selenium, while other sources improved those values numerically. The same trend recorded with increasing Se supplemental levels from 0.15 to 0.30 ppm. These results in the same way of that concerning Se concentration in thigh muscles and liver. In These results is in agreement with those reported by several researchers (Cai *et al.*, 2012; Mei-Sheng *et al.*, 2005; Wang and Xu, 2008; Baowei *et al.*, 2011). Yang *et al.* (2012) reported that serum GSH-Px activity of broilers fed organic selenium diet were 155.83% higher than that in the control chicks fed inorganic selenium diet ($p<0.05$). These results indicate that the effects of organic selenium on enhancing body oxidation resistance were superior to that of inorganic selenium. The improvement of antioxidant status by using Zn-Se-Meth might be due to higher bioavailability of Zn-Se-Meth. Chantiratikul *et al.* (2008) reported increase plasma Se concentration of hens received Zn-Se-Meth compared hens received NaSe in diet. In another research, Wang and Xu (2008) detected increase of GSH-Px activity and TAOC in plasma of broilers fed on diet supplemented with nano-Se at levels between 0.15 and 1.2 mg/kg diet while MDA values were not affected. Huang *et al.* (2003) explained this trend when reported that nano-Se has a size-dependent effect in scavenging various free radicals; small-size nano-Se has greater ability to transfer electrons to radicals. Recently, Wang and Fu (2012) studied the transport and uptake of NaSe, selinomethionine and nano-Se by broiler intestine cells and found that the transportation of selenomethionine and nano-Se from intestine cells were higher than that of sodium selenite and the highest uptake efficiency ($p<0.05$) was observed in cells treated with nano-Se. The reported significant increase of Se concentration in thigh muscles and liver by either increasing supplemental level from 0.15 to 0.30 ppm, or by using organic or nano form of Se was in agreement with results of Zhou and Wang (2011), Hu *et al.* (2012) and Cai *et al.* (2012). Where using nano-Se compared with NaSe in broiler diets. Surai (2006) reported that indeed increase of Se concentration in breast muscles of broilers when organic selenium was at level of 0.20 ppm compared with the same dose of NaSe according to the results of Kricova *et al.* (2003). The same trend of Se concentration in chicken liver or breast muscles was reported by Payne *et al.* (2005) and Pan *et al.* (2007) when adding Se-yeast to broiler diets. The higher determined Se concentration of liver than that of breast muscles was explained previously by Zhou and Wang (2011) who suggested that there is a limit for chicken muscle deposition of Se within a certain range of added nano-Se. At a certain level of nano-Se, selenoenzymes have already been fully saturated and cells may alter to control the Se deposition. However the accumulation of Se in liver of Chinese chicken liver was dose dependent. The overall experimental results were in harmony. The obtained results of growth performance, hematological examination, immunity and antioxidant status were in agreement with the determined Se concentration in liver and thigh muscles.

As shown in Table 4 increasing Se levels supplementation significantly increased T3. The result in agreement with (Preter, 2000) who found that Se deficiency can cause the reduction of T3 synthesis. This may be due to selenium is an important auxiliary factor for the key enzyme of 5,-deiodinase. The iodothyronine deiodinase enzymes convert the pro-hormone thyroxine (T4) to the active form triiodothyronine (T3).

Triiodothyronine is a main hormone that regulates growth by controlling the body's energy and protein anabolism (Arthur *et al.*, 1999; Preter, 2000). In addition, Se may protect the thyroid gland from oxidative damage due to any excess H_2O_2 produced during thyroid hormone synthesis (Arthur *et al.*, 1999). Thus, Se deficiency may exacerbate some effects of I-deficiency and may have a role in the etiology of I-deficiency disorders (Arthur and Beckett, 1999).

The histological examination of liver tissues in this study showed clear adverse effect of high level of Se supplementation (0.30 ppm) in broiler diets compared with adding the lower supplemental level (0.15 ppm). In addition, using Se-Yeast or Zn-Se-Meth was more save to liver tissue compared with inorganic form of Se. Using nano-Se in broiler diets or drinking water caused more deleterious changes in liver tissues, like inflammation and necrosis. These results were matched with those reported by Attia *et al.* (2010) who studied the effect of inclusion inorganic (sodium selenite) or organic (Sel-plex) at levels 0.15 and 0.30 ppm on productive performance and physiological traits of developed Egyptian chicken strain (Gimmizah). They detected milder toxic effects in hepatic tissues by feeding chicks on diets containing organic selenium compared with those detected by feeding on diets containing inorganic form of Se. Furthermore they found that the recorded

changes in liver tissues were dose dependent. In the same trend Benko *et al.* (2012) studied the toxicity of Se sources in mice. They used inorganic sodium selenate and sodium hydroselenite, elementary nano-Se (particle size, 100-500 nm), organic Sel-Plex and Lacto-Micro selenium were administered for 14 d at concentrations of 0.5, 5 and 50 mg Se/kg food. The histological examination of liver tissues indicted that selenate is the most toxic Se compound, followed by selenite. The comparison of the rest of the Se compounds showed that nano-Se was more toxic than Sel-Plex. They reported that this discrepancy could be related to the specificity of the yeast strain, but is more likely to relate to the difference in particle size of nano-Se preparations, indicating that the larger nanoparticles they used could be more toxic than the smaller ones used by others. Indeed, studies related to nano-Se particle size have shown that nano-Se has a size-dependent effect in mice. Seleno compounds accumulate *in vivo* in a size-dependent manner, suggesting that nano-Se is a more effective chemopreventive agent at a smaller nanoparticle size (Peng *et al.*, 2007). These result and explanation shows the importance of using nano-Se in particle size less than 100 nm and following the preparation by characterization of the prepared nano-Se particles before application in the experiment as shown in this study.

According to the results of this study using sodium selenite as Se source in broiler diet caused the worst effects on physiological status and showed more toxic effects on liver tissues and kidney functions especially when added at level 0.30 ppm in diet. Both inorganic and nano forms of Se had more toxicity effects on liver tissues. While using Zn-Se-Meth as organic form of Se or L-Nano Se as nano form of Se or increasing the supplemental Se to 0.30 ppm in broiler diets or its equivalent in drinking water is more effective to get better, physiological, immunological and antioxidant status of broiler chicks, adding Se-Yeast as organic form of Se in broiler diets at level 0.15 ppm was more save to liver tissues and kidney function.

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