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ANTIOXIDANT AND IMMUNOMODULATORY EFFECTS OF NANO-SELENIUM ON RESPONSE OF BROILERS TO ND VACCINE

DALIA M. AZAB [1]; HEMAT S. EL-SAYED [2] and AYMAN S. EL-HABBAA [3]

[1] Biochemistry Department (Pharmacology), Animal Health Research Institute, Benha-Branch, Agriculture Research Center (ARC), Egypt.

[2] Poultry Diseases Department Animal Health Research Institute, Benha-Branch, Agriculture Research Center (ARC), Egypt.

[3] Department of Virology, Faculty of Veterinary Medicine, Benha University

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ABSTRACT

Trace element selenium occupies a privileged position among the natural antioxidant in addition to its ability to enhance the immune response in several species. The current challenges of supplementation of sodium selenite in poultry diets are always linked to low absorption and increased toxicity. Therefore, it is imperative to embrace new technologies like nanotechnology to get as an alternative source of traditional selenium supplementation in form of the nanoparticles with high bioavailability and low toxicity which will be positively reflected on biological activities of selenium. The present study was conducted to investigate the antioxidant and immunomodulatory effects of different concentrations of Nano-selenium on response of broilers to ND vaccination. A total of one hundred and eighty day old Cobb broilers were randomly allocated in 6 groups with each group applied to 3 replicate of 10 chicks, the first two groups were control negative groups without selenium supplementation and the remaining four groups were dietary supplemented with sodium selenite (0.15ppm) and Nano-selenium at concentration of (0.15ppm), (0.075 ppm) and (0.0375 ppm), respectively. The results showed that Nano-selenium had a superior effect compared to that of the sodium selenite either as an antioxidant or as an immunomodulatory and comparing the control negative groups. The most important results can be summarized in the following points; significant improvement in Feed conversion ratio (FCR), significant enhancement in activities of antioxidant enzymes without adverse effects on tested biochemical parameters as well as significant boosting in antibody titers against ND. In conclusion, Nano-selenium supplementation in commercial broilers diets could be an economically a promising multitask option, particularly when it comes to improving antioxidative status and boosting immune response to NDV (LaSota) vaccination in broilers.

***Key words*:** Antioxidant; Broilers; Immunomodulatory; Nano–selenium; ND vaccine

INTRODUCTION

The metabolic processes in the body is produced by-products called the reactive species which included reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Di Meo *et al.*, 2016). The reactive species has a regulatory role in the interactive between host and pathogens including recognition of pathogens, expression and adaptation of genes as well as activation of host defense mechenism. More importantly, the higher percentages of the uncontrolled ROS generation are usually attributed to irreversible damage to proteins, lipids, carbohydrates, and nucleic acids (Aquilano *et al.*, 2014). The control of these reactive species has an important impact on apoptosis (Gloire *et al.*, 2006).

Antioxidant defense systems that prevent damage caused by reactive oxygen species (ROS) during exposure to infections, inflammation, and stressors (Valko *et al.*, 2007) are classified into enzymatic and nonenzymatic components. Enzymatic components include glutathione peroxidase (GSHPx), catalase, superoxide dismutase (SOD), and glutathione reductase, whereas the nonenzymatic system comprises glutathione, thioredoxin, melatonin, carotenoids, vitamin E (VitE), and vitamin C (Ivanov *et al.*, 2016).

Selenium (Se) is one of a potent nutritional antioxidants that plays an important role in regulating reactive oxygen species (ROS). Selenium is an integral component of at least 25 selenoprotein which interfering with many metabolic pathways in the body tissues including Glutathione peroxidase (Brown and Arthur, 2001; Nazıroğlu *et al.*, 2012 and Zhou *et al.*, 2013). Beside its crucial role as an antioxidant, it is consider a very important essential micronutrient that is needed for optimal immune

Corresponding author: Dr. HEMAT.S. EL-SAYED
E-mail address: hemat.elsayed@yahoo.com
Present address: Poultry Diseases Department. Animal Health Research Institute, Benha- Branch, Agriculture Research Center (ARC), Egypt.

responses (Bainbridge, 1976; Behne and Wolters, 1983; Surai and Dvorska, 2001; Gromer *et al*., 2005 and Hoffmann and Berry, 2008) and it was proven that it plays a vital role in enhancing the immune response of the body (Kim and Mahan, 2003; Ghazi *et al.,* 2012; Ebeid *et al.*, 2013 and Habibian *et al.*, 2014). Moreover, selenium is a key element in several compounds that improve the response of immune system either by altering the production of certain cytokines or by strenghtening the immune cells to resist oxidative stress (Habibian *et al.,* 2015).

Inorganic, organic and nano- selenium are the avaliable forms of selenium, the latter form has recently attracted the attention of scientific community due to its unique novel characters exihibited by the fabricated nanoparticles. Nano-selenium appears to be less toxic and more biocompatible compared to sodium selenite in addition to possessing a powerful properities including catalytic efficiency, adsorbing ability, surface activity and chemical stability (Wang *et al.*, 2007; Zhang *et al.,* 2008; Shi *et al.,* 2011 and Boostani *et al.,* 2015 and Skalickova *et al.*, 2017). More importantly, selenium nanoparticles showed better antioxidant capability compared to that of other chemical forms in the bulk state with the advantage of reducing toxicity of selenium (wang *et al.,* 2007).

Previous studies that carried on animals showed that selenium nanoparticles had significant effect on the activities of antioxidant enzymes compared to that of inorganic selenium (Na_2SeO_3). Higher activity of glutathione peroxidase (GPx) was recorded in animals supplemmented with Nano-selenium in their diets (Zhang *et al.,* 2007). Particularly in chicken, previous studies confirmed that dietary Nano-selenium supplementation could have a positive improvment in the antioxidative status of heat-stressed broilers (EL-Deep *et al.,* 2016) and it was effective in enhancing GSH-Px activities in the serum and liver tissue of Guangxi Yellow chicken (Zhou and Wang, 2011). Moreover, dietary nano-Se could enhance the antioxidant ability and oxidative stability in broilers (cai *et al.,* 2012).

With regard to the immunomodulatory role of selenium nanoparticles that assessed in recent studies, it was shown that treatment with administered selenium nanoparticles in tumors bearing mice could be elvate IFN-γ and IL-12 with signifcant induction of the Th1 platform of the immune response (Yazdi *et al.,* 2012). Also selenium nanoparticles could be down regulated of mRNA gene expressions of pro-inflammatory cytokines in a dose-dependent manner as (iNOS), (IL-1), and TNF-α, while marked increase of anti-inflammatory cytokine IL-10 has been reported (Ding *et al.,* 2010 and Wang *et al.,* 2014). Furthermore, dietry Nano-selenium supplementation in brolier showed immunostimulatory effect in chickens (EL-Deep *et al.,* 2016) and appeared to improve the immunity of broiler chicks (Kadhim *et al.,* 2018) along with huge improvement in humoral immunity that was observed in chicken fed on diet containing 0.30 mg/kg of Nano-selenium (Cai *et al.,* 2012).

Newcastle disease (ND) is a continuing problem for poultry industry causing major economic lossess world wide. The caustive agent Newcastle disease virus (NDV) which is known as avian paramyxovirus type 1, belongs to the Avulavirus genus within the Paramyxoviridae (Alexander and Senne, 2008). Oxidative stresses in brain tissue are usually the main character associated with NDV infection in poultry, the virus decreases the activities of glutathione peroxidase (GPx) superoxide dismutase (SOD), and catalase (CAT) (Subbaiah *et al.,* 2011 and 2015). Although vaccination aganist ND is an important strategy employed in endemic countries (Cornax *et al.,* 2012) it does not prevent occurrence of outbreaks with vNDV nor prevents replication of challenge viruses (Choi *et al.,* 2013; Jaganathan *et al.,* 2015). Based on the above stated information and the benefits of nanotechnology application in poultry vaccination to boost immunity, the presented study aimed to clarify the antioxidant and immunomodulatory effects of dietary selenium nanoparticles on response of broilers to ND vaccine.

MATERIALS AND METHOD

Selenium and selenium nanoparticles sources

Sodium selenite ($Na_2\ Se\ O_3.5H_2O$) was purchased from MERCK. Co, Germany. Selenium nanoparticles were prepared by using a chemical reduction of Sodium selenite with ascorbic acid followed by stabilizing the prepared nanoparticles by coating them with Dextrin according to modified method conducted by Malhotra *et al.* (2014). Characterization of prepared selenium nanoparticles including size, shape, morphology and crystallinity was done using different analytical tools including particle spectroscopy, Scanning electron microscope (SEM) and X-ray diffraction (XRD). The size of prepared nanoparticles used in our study was 60nm. Preparation of selenium nanoparticles was carried in Naqaa Foundation for Scientific Research, Technology and Development, Giza, Egypt.

Preparation of experimental diets with sodium selenite and Nano-selenium

Each of sodium selenite $0.15 mg/kg^{-1}$ diet and prepared selenium nanoparticles with concentration of 0.15, 0.075 and 0.0375 mg/kg^{-1} diet, respectively in dietary treated groups was dispersed in 5 mldistilled water then it was mist sprayed onto poultry feed, rotating in mixer and then air dried for 24 hour.

Experimental design

The experiment was performed according to the guidelines of Benha University, Egypt. A total of one hundred and eighty one day- old broiler chicks (Cobb, Al-Watania poultry company) were obtained from a commercial hatchery located in Qalyoubia, Egypt. The chicks were randomly divided into six groups of 30 birds per each (three replicate per group and 10 birds per replicate). After decaying of maternal antibodies at 21 days old, all birds in all groups except group no. 1 were received ND vaccination with Lasota strain via drinking water (Servac, Egypt). The dietary treatments of the current study were as following: birds in groups1 and 2 fed on basal diet without Se supplementation; birds in group3 fed on basal diet with 0.15 mg of sodium selenite [Na_2SeO_3] per kilogram of diet while birds in groups 4,5 and 6 were fed on basal diet with 0.15 mg, 0.075mg and 0.0375mg of prepared selenium nanoparticles per kilogram of diet, respectively. Feed was ad-libitum for birds. The basal diet was formulated according to National Research Council (NRC, 1994) recommendations for all other nutrients to meet the nutritional requirements of growing broilers as shown in table1.

Table 1: Composition of the basal diets.

Ingredient %	Starter up to 21 days	Grower from 22 to 42 days
Corn grain	53.30	64.725
Soya bean 44% protein	30.30	15.4
Concentrate	10.0	10.0
Corn gluten	2.20	6
Vegetable oil	3.0	2.5
Di-calcium phosphate	0.65	0.5
DL-Methionine	0.22	0.025
L-Lysine	0.15	0.3
Threonine	0.135	0.09
Sodium chloride	0.11	0.1
Lime stone	0.05	0.05
Chemical analysis of basal diets	**Starter**	**Grower**
Crude protein	22.480	20.04
ME (Kcal/Kg diet)	3051.5	3201.9
Calcium	1.04	1.01
Phosphorus(%) (available)	0.50	0.45
Lysine(%)	1.38	1.15
Methionine (%)	0.7	0.5
Methionine + Cysteine (%)	0.73	0.84
Determination of selenium in experimental diets (mg/kg)		
Group 1 without Se supp.	0.005	
Group2 without Se supp.	0.005	
Group3 supp. with 0.015 inorganic Se	0.147	
Group4 supp. with 0.015 Nano- Se	0.152	
Group5 supp. with 0.075 Nano- Se	0.071	
Group6 supp. with 0.0375 Nano- Se	0.038	

Supp. supplementation , Se. selenium

The experimental diet was formulated in accordance with recommendations of National Research Council (NRC, 1994). Food Analysis Center, Faculty of veterinary medicine, Benha University performed the chemical analysis of diets.

Growth Performance parameters

Body weights and feed consumption per group were recorded weekly and the obtained values were used to calculate weight gain and Feed conversion ratio for each group expressed according to Wanger *et al.* (1983) as follows

$$\textbf{FCR} = \frac{\text{Average feed intake (g) bird/week}}{\text{Average body weight gain (g) bird/week}}$$

Sampling

Blood samples of five birds from each replicate per group were collected at weekly intervals from one day-old to day 42 of age for estimating maternal antibody and evaluating of humoral immunity on response to ND vaccine. For determination of serum biochemical and serum antioxidant parameters, blood samples of two birds from each replicate per group were collected at day 22 and 42 days of age. The samples were immediately transferred into sterile test tubes and serum was harvested. The serum was stored at −20°C for later analysis of antioxidant and immune parameters.

Biochemical parameters analysis:
Activities of alanine aminotransferase(ALT), aspartate aminotransferase (AST) enzymes, total protein, albumin, uric acid, creatinine, total cholesterol in tested sera were determined using the spectrophotometric method (RAL, Barcelona, Spain.) and Bioanalytica test kits (Bioanalitika doo, Beograd, Serbia) as described by (Rej and Hoder, 1983). Serum globulin (G) was calculated as follows: G = total protein – albumin.

Determination of antioxidant indices:
The SOD, GSH-Px activities and MDA content were determined by ELISA kits produced by Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to Dalton *et al.* (2000).

Haemagglutination inhibition assay (HI)
The HI test was performed on tested chicken sera in V-bottomed microwell plates according to the OIE Manuel (OIE, 2012). Four HA unit of NDV antigen (Lasota, Servac, Egypt) were used. Negative and positive control sera were used to confirm the test results. The HI titer was determined as the reciprocal of the highest dilution of serum that caused total inhibition of HA activity with 4 HA unit of antigen. The test for each serum sample were repeated in duplicate. The HI titer were expressed as the reciprocal of log2. Tested sera were considered as positive sera with HI titers ≥ 4 according to (OIE, 2012). Geometric mean titers (GMT) were calculated and cumulative mean titers (CMT) per each group were measured weekly during experimental period.

Statistical analysis:
The data were analyzed by determine the normality via Shapiro-Willk test. The differences between groups were analyzed by One-Way ANOVA followed by Duncan's multiple comparison Post Hoc tests (Duncan, 1955). The Statistical Package for Social Science (SPSS Inc. Released, 2009) (version 20.0; SPSS Inc., Chicago IL, USA) was used for performing the statistical analyses to determine difference between groups. Significance between mean values was set a statistically at $P<0.05$.

RESULTS

Growth performance parameters (Body Weights and Feed conversion ratio FCR):
In the first week there is no significant differences across the treatment groups. However, significant differences between groups ($P < 0.05$) were observed starting from the second week (Table,2). Higher body weights were observed in group 4 which supplemented with Nano-selenium 0.15ppm compared with the control groups, on the other hand group 3 supplemented with sodium selenite 0.15ppm that showed lower body weights compared with control groups. FCR were significantly improved ($P<0.05$) in group supplemented with Nano-selenium 0.15ppm at weekly interval compared to control and other dietary treated groups as shown in table 3.

Table 2: Effect of inorganic selenium and Nano-selenium supplementation on body weights in broilers.

Dietary treatment	Estimated body weights (g) /chick (mean± SE)						
	Zero day	1st week	2nd week	3rd week	4th week	5th week	6th week
NVG-no Selenium supp.	**46.33 ±0.67a**	**166.67 ±10.48a**	**302.00 ±0.67ab**	**567.83 ±11.29b**	**993.1 ±6.15ab**	**1296.67 ±23.52b**	**1682.33 ±29.53b**
VG- no Selenium supp	**45.67 ±0.33a**	**164.67 ±5.84a**	**301.00 ±0.33ab**	**535.63 ±10.79bc**	**931 ±11.20b**	**1309.7 ±14.59b**	**1629 ±17.78bc**
VG-T Sodium selenite 0.15 ppm	**47.67 ±1.20a**	**160.67 ±4.25a**	**274.67 ±1.20b**	**514.33 ±24.63c**	**942 ±26.13b**	**1140.6 ±22.98c**	**1590 ±17.32c**
VG –T Nano-Se 0.15 ppm	**46.33 ±0.33a**	**166.67 ±1.76a**	**301.33 ±0.33ab**	**635.53 ±8.80a**	**1068 ±51.62a**	**1404.4 ±22.24a**	**1823.67 ±23.95a**
VG –T Nano-Se 0.075 ppm	**48.00 ±1.00a**	**164.33 ±6.12a**	**324.66 ±1.00a**	**544.17 ±15.25bc**	**899 ±7.89b**	**1290.7 ±19.05b**	**1869.3 ±33.05a**
VG-T Nano-Se 0.0375 ppm	**46.0 0±1.00a**	**166.33 ±2.73a**	**294.33 ±1.00b**	**528.83 ±12.27bc**	**918 ±41.37b**	**1254.4 ±41.81b**	**1820.7 ±38.49a**

NVG, nonvaccinated group; VG, vaccinated group, T treated with; Supp. Supplementation. a,b,c, mean values with different superscripts in a column are statistically different at ($p< 0.05$)

Table 3: Effect of inorganic selenium and Nano-selenium supplementation on feed conversion ratio (FCR) in broilers.

Dietary treatment	Feed conversion ratio (FCR) (mean± SE)					
	1st week	2nd week	3rd week	4th week	5th week	6th week
NVG-no Selenium supp.	**1.26 ±0.11ab**	**2.56 ±0.19a**	**1.68 ±0.07b**	**1.86 ±0.05ab**	**2.31 ±0.21ab**	**2.39 ±0.09a**
VG- no Selenium supp	**1.27 ±0.07ab**	**2.21 ±0.13a**	**1.93 ±0.09ab**	**1.72 ±0.03b**	**2.26 ±0.09ab**	**2.14 ±0.10b**
VG-T Sodium selenite 0.15 ppm	**1.43 ±0.40a**	**2.60 ±0.14a**	**1.85 ±0.10b**	**1.67 ±0.11b**	**2.54 ±0.13a**	**2.40 ±0.03a**
VG –T Nano-Se 0.15 ppm	**1.12 ±0.02b**	**1.50 ±0.03b**	**1.37 ±0.06c**	**1.33 ±0.13c**	**1.98 ±0.15b**	**2.08 ±0.07b**
VG –T Nano-Se 0.075 ppm	**1.28 ±0.06ab**	**1.61 ±0.09b**	**1.68 ±0.08b**	**2.08 ±0.09a**	**2.23 ±0.07ab**	**2.12 ±0.09b**
VG-T Nano-Se 0.0375ppm	**1.26 ±0.02ab**	**2.21 ±0.11a**	**2.17 ±0.05a**	**1.85 ±0.16ab**	**2.25 ±0.05ab**	**2.09 ±0.04b**

NVG, nonvaccinated group; VG, vaccinated group, T treated with; Supp. Supplementation. a,b,c, mean values with different superscripts in a column are statistically different at ($p < 0.05$)

Antioxidant enzymes of broilers at 22 and 42 days of age:

The results of antioxidant enzyme assays are given in Table (4). In general there was a significant ($P < 0.05$) increase in GPX and SOD with significant decrease in MDA in groups treated with Nano-selenium compared to vaccinated group treated with inorganic Se.

Briefly, GPX level in examined sera at 22 days in treated group VGT2 (Nano-Se 0.15 ppm) showed significant increased compared to all groups. The highest activity of GPX was observed at 42 days of age in VGT2 and VGT3 (VG –T Nano-Se 0.15 and 0.075 ppm) respectively compared to NVG, VG, VGT1 and VGT4.

Regarding SOD activity, there was a significant increase in VGT2 and VGT3 compared to all groups at 42 days of age, while VGT2 showed the highest level with significant difference compared to other groups at 22 days of age.

The lowest level of MDA in serum appeared in VGT2 (VG –T Nano-Se 0.15 ppm) compared to the other groups at 42 days of age, while MDA level in VGT1 was significantly decreased compared to NVG and VG. At 22 days of age VGT1, VGT2 and VGT3 were decreased compared to NVG.

The GPX and SOD were improved in response to selenium nanoparticles 0.15 and 0.075 ppm ($P < 0.05$) compared to inorganic source of Se. The lowest MDA level in serum was observed in vaccinated group treated with Nano Se (VGT2 0.15 ppm). According to contrast comparisons, there were significant differences between sodium selenite versus selenium nanoparticles in GPX, SOD and MDA in serum. This indicates that selenium nanoparticles (0.15 ppm) can be used instead of inorganic sources of Se (0.15 ppm) to produce higher activity level of GPX, SOD and lower MDA level in broiler chicks.

Blood biochemical parameters of broilers at 22 and 42 days of age:

Evaluation of dietary sodium selenite and Nano-selenium at different concentrations in the different groups on blood biochemical parameters are shown in Tables (5,6).

Dietary supplementation of sodium selenite and different concentration of selenium nanoparticles does not change serum total protein, albumin and globulin at 22 and 42 days of age. In spite of that, the differences were significant ($P < 0.05$) between the groups in serum cholesterol, AST, ALT, creatinine levels and uric acid.

AST and ALT levels in serum were decreased in birds fed Nano-selenium compared to inorganic Se (VGT1). ALT level was significantly decreased ($P < 0.05$) in VGT2 compared to NVG at 22 days of age and also decreased in all groups treated with Nano-selenium compared to NVG and VGT1 at 42 days of age. AST level was significantly lower in VGT2 and VGT3 than VGT1 at 22 days of age and also lower than NVG and VG at 42 days of age. In addition, Uric acid level in VGT3 (0.075ppm) was decreased compared to NVG at 42 days of age, while no significant differences were showed at 22 days of age. Moreover, dietary Nano-seleniumVGT2 (0.15ppm) decrease the serum creatinine level compared to all groups at 22 days of age, while at 42 days of age the difference in VGT2 was decreased significantly compared to NVG, VG, and VGT3 while no significant changes were observed compared to VGT1 and VGT4. Cholesterol level was lower in VGT2 than all groups at 22 days of age, while no differences were showed compared to VGT1, but the level was decreased in VGT2 than NVG, VG, VGT3 and VGT4 at 42 days of age.

Table 4: Antioxidant parameters of inorganic selenium and different concentrations of Nano-selenium in serum of broilers at 22 and 42 days of age (Mean*±SE).

Dietary treatment	Antioxidant parameters					
	GPX (ng/ml)		MDA (nmol/ml)		SOD (U/L)	
	22 day	42 day	22 day	42 day	22 day	42 day
NVG-no Selenium supp.	**0.125 ±0.017[c]**	**0.136 ±0.018[c]**	**0.458 ±0.022[a]**	**0.401 ±0.019[a]**	**0.102 ±0.011[c]**	**0.092 ±0.01[c]**
VG- no Selenium supp.	**0.139 ±0.016[c]**	**0.164 ±0.013[c]**	**0.418 ±0.018[ab]**	**0.399 ±0.025[a]**	**0.183 ±0.019[b]**	**0.157 ±0.015[c]**
VG-T1 Sodium selenite 0.15 ppm	**0.185 ±0.011[bc]**	**0.263 ±0.015[b]**	**0.372 ±0.035[b]**	**0.304 ±0.026[b]**	**0.205 ±0.013[b]**	**0.299 ±0.018[b]**
VG –T2 Nano-Se 0.15 ppm	**0.326 ±0.044[a]**	**0.415 ±0.012[a]**	**0.339 ±0.030[b]**	**0.231 ±0.018[c]**	**0.335 ±0.033[a]**	**0.447 ±0.023[a]**
VG –T3 Nano-Se 0.075 ppm.	**0.229±0.016[b]**	**0.385± 0.021[a]**	**0.350±0.021[b]**	**0.272 ±0.020[bc]**	**0.244± 0.023[b]**	**0.432 ±0.039[a]**
VG-T4 Nano-Se 0.0375ppm.	**0.175±0.030[bc]**	**0.244± 0.017[b]**	**0.407±0.025[ab]**	**0.311 ±0.029[b]**	**0.186± 0.027[b]**	**0.249 ±0.027[b]**

NVG, non-vaccinated group; VG, vaccinated group, T treated with; Supp. Supplementation. a,b,c, mean values with different superscripts in a column are statistically different at ($p < 0.05$) ; *mean of 6 birds.

Table 5: Effect of inorganic selenium and Nano-selenium supplementation on blood biochemical parameters in broilers at 22 days of age (Mean*±SE).

Dietary treatment	**Blood biochemical parameters at 22 days**							
	ALT(U/L)	**AST(U/L)**	**Total protein (gm/dl)**	**Albumin (gm/dl)**	**globulin (gm/dl)**	**Uric acid (mg/dl)**	**Creatinine (mg/dl)**	**Total Cholesterol (mg/dl)**
NVG-no Selenium supp.	**25.19 ±1.02ab**	**178.90 ±2.36a**	**3.82 ±0.068ab**	**1.58 ±0.111a**	**2.24 ±0.128a**	**4.97 ±0.432ab**	**0.35 ±0.015a**	**156.77 ±2.83a**
VG- no Selenium supp.	**26.81 ±1.21a**	**172.70 ±3.03a**	**3.47 ±0.18b**	**1.73 ±0.166a**	**1.74 ±0.338a**	**5.71 ±0.217a**	**0.32 ±0.018a**	**156.18 ±2.99ab**
VG-T1 Sodium selenite 0.15 ppm	**25.20 ±0.89ab**	**174.22 ±2.24a**	**3.71 ±0.258ab**	**1.64 ±0.18a**	**2.07 ±0.341a**	**4.51 ±0.202b**	**0.34 ±0.016a**	**148.57 ±3.52bc**
VG –T2 Nano-Se 0.15 ppm	**22.97 ±0.69b**	**161.42 ±3.25b**	**4.04 ±0.41ab**	**1.66 ±0.128a**	**2.39 ±0.438a**	**4.16 ±0.232b**	**0.27 ±0.020b**	**132.30 ±1.66e**
VG –T3 Nano-Se 0.075 ppm	**24.29 ±1.03ab**	**157.19 ±2.98b**	**3.44 ±0.23b**	**1.94 ±0.219a**	**1.50 ±0.187a**	**4.29 ±0.46b**	**0.33 ±0.015a**	**140.13 ±2.53d**
VG-T4 Nano-Se 0.0375ppm	**25.56 ±0.43ab**	**171.62 ±3.21a**	**4.39 ±0.37a**	**1.96 ±0.131a**	**2.44 ±0.380a**	**4.25 ±0.38b**	**0.34 ±0.015a**	**143.30 ±2.29cd**

NVG, non-vaccinated group; VG, vaccinated group, T treated with; Supp. Supplementation. a,b,c, mean values with different superscripts in a column are statistically different at ($p < 0.05$) ; *mean of 6 birds

Table 6: Effect of inorganic selenium and Nano-selenium supplementation on blood biochemical parameters in broilers at 42 days of age (Mean*±SE).

Dietary treatment	Blood biochemical parameters at 42 days							
	ALT(U/L)	AST(U/L)	Total protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Total Cholesterol (mg/dl)
NVG-no Selenium supp.	27.18 ±0.829a	185.13 ±2.04a	4.08 ±0.278a	1.96 ±0.061a	2.13 ±0.29ab	3.98 ±0.347a	0.32 ±0.016a	159.18 ±2.47a
VG- no Selenium supp.	24.51 ±0.749bc	177.69 ±2.05b	4.43 ±0.199a	1.90 ±0.064a	2.53 ±0.151a	3.80 ±0.184ab	0.33 ±0.010a	160.42 ±2.95a
VG-T1 Sodium selenite 0.15 ppm.	26.76 ±0.993ab	170.59 ±2.28cd	4.49 ±0.283a	1.96 ±0.165a	2.52 ±0.241a	3.31 ±0.213ab	0.29 ±0.015ab	127.83 ±1.77d
VG -T 2Nano-Se 0.15 ppm.	23.56 ±0.702c	165.46 ±2.32d	4.04 ±0.32a	2.04 ±0.195a	1.99 ±0.363ab	3.85 ±0.402ab	0.25 ±0.018b	128.87 ±1.48d
VG -T3 Nano-Se 0.075 ppm.	22.39 ±0.642c	175.64 ±2.36bc	3.55 ±0.350a	2.15 ±0.231a	1.41 ±0.394a	2.99 ±0.171b	0.30 ±0.017a	135.3 ±1.91c
VG-T4 Nano-Se 0.0375ppm.	24.21 ±0.778c	180.24 ±2.39ab	4.16 ±0.294a	1.89 ±0.129a	2.27 ±0.359ab	3.40 ±0.202ab	0.29 ±0.015ab	146.50 ±1.78b

NVG, non-vaccinated group; VG, vaccinated group, T treated with; Supp. Supplementation. a,b,c, mean values with different superscripts in a column are statistically different at ($p< 0.05$) ; *mean of 6 birds,

Immunomodulatory effect of different dietary treatments on HI titers against ND vaccination.
In the present study we evaluate the effect of dietary Nano-selenium at different concentrations in the different groups regarding response to ND vaccination in broiler chicks by HI test. Our results revealed that, supplementation of Nano-selenium with concentration of 0.15 ppm and 0.075 ppm in groups 4 and 5, respectively showed significantly boost (P<0.05) in their HI titers compared to that of vaccinated control group (2) at weekly interval post vaccination. On the other hand group 3 which supplemented with inorganic selenium showed no boost effect (figure 1).

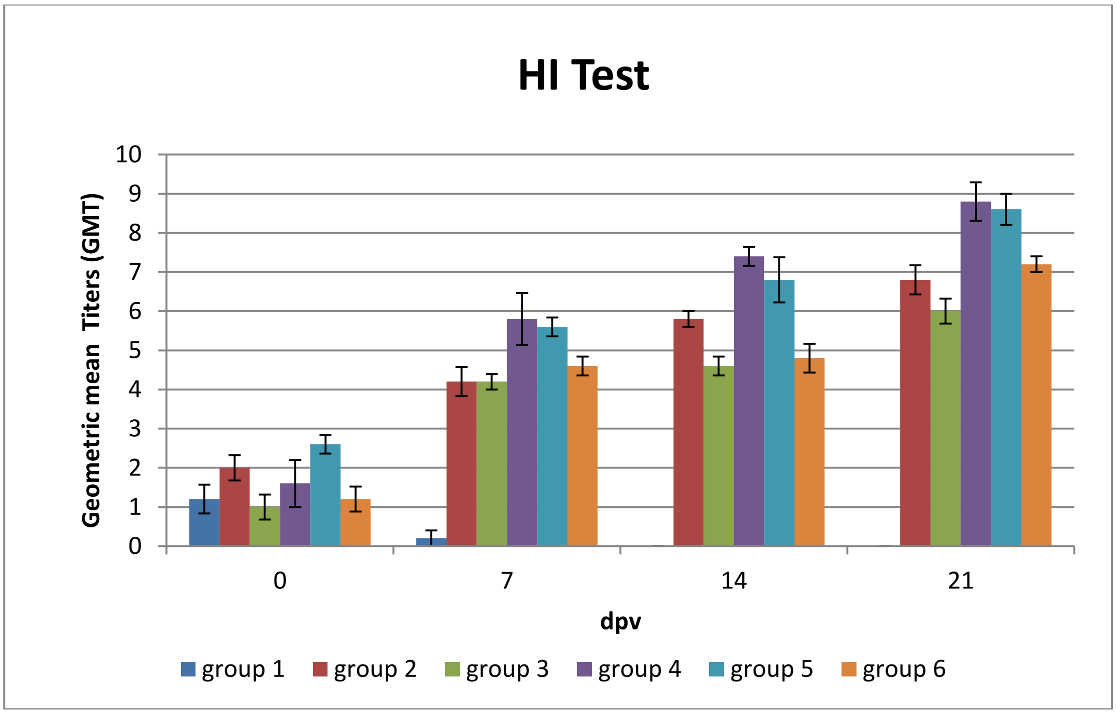

Fig. 1: Effect of inorganic selenium and Nano-selenium supplementation on antibody titers against ND vaccination in broiler chicks.

dpv, days post vaccination, group1, non-vaccinated non treated, group 2 vaccinated non treated, group 3, vaccinated dietary treated with inorganic selenium (0.15ppm), group 4 vaccinated dietary treated with Nano-selenium (0.15 ppm), group 5 vaccinated dietary treated with Nano-selenium (0.075 ppm), group 6 vaccinated dietary treated with Nano-selenium (0.0375 ppm).

DISCUSSION

Currently, the supplementation of poultry ration with selenium is limited to the inorganic form (sodium selenite) or selenium containing organic compound (Mohapatra *et al.,* 2014). Following the results of several studies that confirmed the higher bioavailability and less toxicity of Nano-selenium compared to sodium selenite in different animal species including mice, rat, goat and broilers (Jia *et al.,* 2005; Zhang *et al.*, 2005, Wang *et al.*, 2007; Shi *et al.*, 2011 and Zhou and Wang, 2011). Scientists were encouraged to study the impact of dietary Nano-selenium as a good alternative choice for other traditional sources of bulk selenium used in poultry ration. The most previous studies associated with supplementation of Nano-selenium in broilers diets showed that there is no significant effect of Nano-selenium on growth performance and feed conversion (Cai *et al.*, 2012, EL-Deep *et al.,* 2016 and Gangadoo *et al.*, 2017). Indeed, our results clearly indicated that body weights and FCR were significantly improved ($P<0.05$) when broiler birds were given diets supplemented with Nano-selenium 0.15 ppm compared to other dietary treatments which showed no significant improvement in comparison with control groups. These results agreed with those obtained by Zhou and Wang, (2011) who reported that, FCR was significantly improved when birds were given diets with Nano-Se. Also, Jayanthi *et al.* (2018) reported that, improvement of growth performance of broilers with reduction in production cost was achieved when broilers were fed on a diet containing 0.11 mg/kg of Nano-Se (75% of inorganic Se). In our study Nano-selenium at concentration (0.15 ppm) could improve the body weights and FCR compared to the same concentration of inorganic selenium which failed to improve the growth performance parameters. These differences might be attributed to the high absorption of Nano-selenium from the intestinal lumen into the body compared to that of inorganic selenium (sodium selenite) (Hu *et al.,* 2012). These results agreed with EL-Deep *et al.* (2016) who showed the superiority of Nano-selenium compared to sodium selenite in broiler chickens regarding its efficacy and bioavailability.

Animal nutrition is important factor in animal antioxidant system. The up-regulation process of Se-containing antioxidant enzymes and glutathione pool in the body were greatly affected by selenium supplementation in animal diets (Jiang *et al.*, 2009). Some literatures referred to the role of selenium in activating GSH-Px was reflected positively on the improvement of the antioxidant status (Ebeid *et al.*, 2013). Both of Glutathione peroxidase and superoxide dismutase are considered an essential antioxidants that play an important role in combating toxic oxygen reduction metabolites (Zhang *et al.*, 2014). Herein, one of the important points in our results is the significant ($p<0.05$) increase in GPX and SOD activities in group supplemented with Nano-selenium 0.15 mg/kg diets compared to those supplemented with sodium selenite with the same concentration. These results agree with Aparna and Karunakaran (2016) who observed an increase in SOD and glutathione peroxidase cellular activity in birds fed with (0.1875 mg/kg) selenium nanoparticles when compared to control group and other treatment groups. Also Cai *et al.* (2012) showed an increase in activity of glutathione peroxidase in both serum and tissue in groups supplemented with Nano-selenium compared to the control group.

Oxidative stress marker, Malondialdehyde is one of the final products of cell polyunsaturated fatty acid peroxidation. In a previous study carried by Gaweł *et al.* (2004), they showed that organic selenium can decrease the level of MDA as well as decrease the production of lipid peroxidation products compared to that of sodium selenite and they attributed to the high bioavailability of organic selenium. In the present study, MDA levels in serum were significantly decreased in vaccinated group supplemented with Nano-selenium (0.15ppm) compared to the same concentration of sodium selenite (0.15ppm). These results agreed with El-Deep *et al.* (2016) who showed that Nano-selenium in broiler diets could enhance the activities of GSH-Px, SOD and CAT in addition to reducing MDA content in the liver and muscles of broilers.

Referring to the results of biochemical parameters tested in broilers sera in our study, both sodium selenite and Nano-selenium had no effect on serum total protein, albumin and globulin compared to control groups. These results agreed with those recorded by Yang *et al.* (2012). ALT and AST enzymes currently used as indicators of the oxidative damage of liver. The reduction in the levels of ALT, AST, urea and creatinine enhance the protection odds against oxidative damage. Moreover, higher blood cholesterol level was used as an index for stressful situations (Boostani *et al.*, 2015). In this study, dietary Nano-Se (0.15 and 0.075ppm) significantly decreased the levels of ALT, AST, urea, cholesterol and creatinine in broilers sera. These results supported by findings obtained by Dalia *et al.* (2017) who reported reduction of AST, ALT and creatinine levels in sera of broilers fed on Nano-selenium in their diets. Moreover, our results agreed with Mohapatra *et al.* (2014) who reported that Nano-selenium in diets of layer chicks could significantly decrease ($P < 0.05$) the levels of total cholesterol and triglyceride. Our results indicated that Nano-selenium had a broad range of safety compared to that of sodium selenite. These findings agreed with the results of previous studies carried on broilers that referred to the low toxicity and higher bioavailability of Nano-selenium compared to sodium selenite (Hu *et al.*, 2012).

ND is one of the most important diseases causing high economic losses in poultry sectors. Vaccination against ND is a routine and regular regime applied in all chicken farms in Egypt to provide the protection against the disease. Vaccine failure is one of common recurring problems in commercial poultry flocks, among the different reasons of vaccine failure which occupy the forefront is the nutritional cause where it provide the optimum condition for development and function of immune system (Khan *et al.,* 1993). The dietary selenium is an effective element needed for optimum development and function of the immune system (Koski and Marilyn, 2003). Humoral immunity is greatly influenced by insufficient concentration of selenium in diets where low levels of IgG and IgM antibodies associated with selenium deficiency were recorded in some studies (Suchý *et al.*, 2014). Referring to the results of previous study conducted by Funari *et al.* (2012) that showed there is no effect of selenium in both available forms (in- organic and organic) on humoral immunity of broilers on the response against Newcastle vaccine. Our results in the present study agreed with those obtained by Funari *et al.* (2012) at particular point which is: the inorganic selenium Na_2SeO_3 in vaccinated treated group 3 showed no effect on improvement of humoral immunity of broilers against the vaccine of Newcastle disease. More importantly results in our study showed that, dietary treatments with selenium nanoparticles at concentration of 0.15 ppm and 0.075 ppm in treated groups 3 and 4, respectively showed significant improvement ($P<0.05$) in the humoral immunity of broiler against the vaccine of Newcastle disease at 7,14 and 21 days post vaccination compared to vaccinated non treated group (2). These results agree with those of Cai *et al.* (2012) who reported that, dietary supplementation of Nano-Se in broiler ration resulted in improving the humoral immunity. Moreover they found that the levels of IgG and IgM ($P< 0.01$) were highest in broilers fed on diet supplemented with 0.30 mg/kg of Nano-Se, while our study found that, 0.15 mg/kg and 0.075 mg/kg of Nano-Se exhibited the highest antibody titer at weekly interval post vaccination ($P<0.05$) which was a good opportunity to reduce the economic cost of Nano-selenium. Another point of interest, selenium nanoparticles possess superior effect on humoral immunity in comparison to inorganic selenium (Na_2SeO_3) and this was an excellent chance to take advantage of selenium nanoparticles with reduced selenium toxicity.

CONCLUSION

Incorporation of new technologies applications particularly nanotechnology could be a promising solution for current challenges in poultry nutrition. The present study suggested that Nano-Se could be used in broilers ration as an alternative source instead of traditional sources of selenium. Our results showed that Nano-selenium could improve growth performance and immunity as well as enhance the antioxidative status in broilers.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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التأثيرات المضادة للأكسدة والمناعية للنانوسيلنيوم على استجابة دجاج التسمين للقاح النيوكاسل

داليا محفوظ عزب ، همت شفيق السيد ، أيمن سعيد الهباء

E-mail: hemat.elsayed@yahoo.com Assiut University web-site: www.aun.edu.eg

يحتل عنصر السيلنيوم مكانة متميزة بين مضادات الأكسدة الطبيعية بالإضافة إلى قدرته على تعزيز الاستجابة المناعية في العديد من السلالات. غالبا ما ترتبط التحديات المتعلقة بإضافة السلينيوم في علائق الدواجن بقلة الامتصاص وزيادة السمية. لذلك ، من الضروري احتضان تكنولوجيات جديدة مثل تقنية النانو تكنولوجي للحصول على بديل لمصادر السيلينيوم التقليدية في شكل جسيمات نانوية ذات اتزان بيولوجي مرتفع وسمية منخفضة ، والتي سوف تنعكس بشكل إيجابي علي الأنشطة البيولوجية للسيلينيوم. أجريت الدراسة الحالية للتحري عن التأثير المضاد للأكسدة والتأثير المناعي لتركيزات مختلفة من النانو سيلينيوم في دجاج التسمين على الاستجابة لتحصين النيوكاسل. تم توزيع عدد مائة وثمانين من دجاج التسمين (كب) في ٦ مجموعات على ان تقسم كل مجموعة إلى ٣ مكررات من ١٠ فراخ ، وكانت المجموعتان الأوليتان سالبة دون إضافة السيلينيوم في العليقة بينما تم إضافة الصوديوم سيلنيت بنسبة (٠,١٥) والنانو سيلينوم بنسبة (٠,١٥) ، (٠,٠٧٥) و (٠,٠٣٧٥) جزء في المليون في علائق الدجاج للمجموعات الأربعة المتبقية ، على التوالي. أظهرت النتائج أن النانو سيلينيوم كان له تأثير متفوق عن الصوديوم سيلنيت سواء كمضاد للأكسدة أو مناعي وبالمقارنة مع المجموعتين السالبتين. أهم النتائج تتلخص في النقاط التالية ، تحسن معنوي في معدل التحويل الغذائي ، تعزيز معنوي في أنشطة أنزيمات مضادات الأكسدة دون آثار ضارة على القياسات البيوكيميائية المختبرة وكذلك زيادة معنوية في تترات الأجسام المضادة ضد النيوكاسل. نستخلص من هذه الدراسة أنه ، يمكن أن تكون مكملات النانوسيلينيوم في علائق دجاج التسمين خيارًا واعدًا متعدد المهام من الناحية الاقتصادية ، لا سيما عندما يتعلق الأمر بتحسين وضع مضادات الأكسدة وتعزيز الاستجابة المناعية للقاح اللاسوتا في دجاج التسمين.