aJava

Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Animal and Veterinary Advances 9 (10): 641-652, 2014
ISSN 1683-9919 / DOI: 10.3923/ajava.2014.641.652

Effects of Dietary Nano-Selenium Supplementation on the Performance of Layer Grower Birds

[1]P. Mohapatra, [1]R.K. Swain, [1]S.K. Mishra, [2]T. Behera, [2]P. Swain, [3]N.C. Behura, [4]G. Sahoo, [1]K. Sethy, [5]B.P. Bhol and [6]K. Dhama

[1]Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, 751003, Odisha, India

[2]Fish Health Management Division, Central Institute of Freshwater Aquaculture, Bhubaneswar, 751002, Odisha, India

[3]Department of Poultry Science,

[4]Department of Veterinary Biochemistry,

[5]Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, 751003, Odisha, India

[6]Division of Pathology, Indian Veterinary Research Institute, Izatnagar, Uttar Paradash, 243122, India

Corresponding Author: R.K. Swain, Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, 751003, Odisha, India

ABSTRACT

To study the comparative effects of Nano Selenium (Nano-Se) and sodium selenite on the growth, bioavailability, antioxidative activities, hematological and biochemical parameters, cellular and humoral immunity, a trial was conducted on BV 300 layer birds during grower phase (9-20 after weeks) in six treated groups. After twenth weeks body weight of the all Nano-Se treated groups (up to a dose of 0.3 mg kg^{-1} of diet) was found to be significantly ($p<0.05$) higher than sodium selenite treated and control groups. However, further increase in dietary Nano-Se content in feed had negative effect on body weight of bird. Birds fed with both Nano-Se and sodium selenite showed higher ($p<0.05$) Se content in different tissues (Such as breast muscle, liver, kidney, pancreas, serum and feathers). However, Se content in liver, breast muscle, pancrease and feathers were signifcantly higher ($p<0.05$) in Nano-Se treated groups. In addition, significant ($p<0.05$) difference was observed as regard to glutathione peroxidase (GSH-Px), erythrocyte catalase (CAT) and superoxide dismutase (SOD) activities among the treated groups. Significantly better cellular and humoral immunity response were observed in Se supplemented birds. Dietary supplementation of Nano-Se improved the body weight, feed consumption ratio, antioxidant status, immunity and tissue Se deposition in grower birds.

Key words: Nano-Selenium, antiradical, biochemical, bioavailability, haematology, immunity, grower birds

INTRODUCTION

Nanotechnology is one of the branches of science that includes design, characterization, production and application of structures, devices and systems by controlling shape and size at nanometer scale. With the use of nanotechnology, nanoparticles can be used as a supplemental source of trace minerals in diets. The rapid development of nanotechnology holds great promises for application in medicinal and nutritional science because nano-materials have been found to have several novel properties different to those bulk materials.

Dietary selenium is an essential trace element for animals and humans with a variety of biological functions (Surai, 2006). Selenium is necessary for growth, fertility, immune system, hormone metabolism, cell growth and antioxidant defence systems in animals and humans (Pappas and Zoidis, 2012). Se-deficiency in poultry causes exudative diathesis, pancreatic dystrophy and nutritional muscle dystrophy of the gizzard, heart and skeletal muscle (Cantor *et al*., 1982). Additionally insufficient immunity, lowering of production ability, lower fertility and laying capacity, decreased feathering of chickens and increased embryo mortality may occur due to selenium deficiency. Therefore, poultry diets require supplemental Se in order to provide a margin of safety against deficiency and to maintain productive performance. Both organic and inorganic forms of selenium are used as supplements in the poultry diet. Subsequent studies report that nano-elemental Se possesses comparable efficiency with other Se sources (Zhang *et al*., 2005, 2008).

Recently, nano elemental selenium (Nano-Se) which is bright red, highly stable, soluble and of nano meter size in the redox state of zero (Se^0), has attracted wide spread attention due to its high bioavailability and low toxicity. Because nanometer particulates exhibit novel characteristics such as great specific surface area, high surface activity, high catalytic efficiency and strong adsorbing ability (Zhang *et al*., 2001). However, little has been done to study the effect of the novel Nano-Se in layer grower birds. The studies on Nano-Se supplementation are only few in number and the findings are rather inconsistent. Thus, the purpose of this experiment is to study effects of dietary Nano-Se on growth performance, tissue deposition, antioxidant defence system and immune functions in layer grower birds in comparison to sodium selenite.

MATERIALS AND METHODS

Selenium sources: Nano red elemental selenium particles (Nano-Se) were synthesized as per the method described by Zhang *et al*. (2001). One milliliter of 25 mM sodium selenite was mixed with 4 mL of 25 mM GSH containing 15 mg of BSA for the Nano-Se preparations. The pH of the mixture was adjusted to 7.2 with 1.0 M sodium hydroxide, forming red elemental Se and oxidized GSH. The red suspension was dialyzed against double-distilled water for 96 h, with the water being changed every 24 h to separate the oxidized GSH from the Nano-Se. The final suspension containing Nano-Se and BSA was lyophilized and stored at room temperature. The size of the red elemental Se was 50-100 nm as determined by Dynamic Light Scattering (DLS) analysis using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) with the average size being 80 nm. Sodium selenite (Na_2SeO_3) was purchased from Sigma-Aldrich Co., USA.

Experimental birds: Commercial (BV 300) layer chicks (n = 300) were randomly allocated to six dietary treatments. Each treatment group had 2 replicates containing 25 chicks in each replicate. Earlier in the first phase, the experiment continued till 8th week of age (starter phase) (Mohapatra *et al*., 2014). This was a continuous study of the same treatment groups. The grower birds were provided 24 h free access to clean drinking water.

Experimental diets: Basal diet was formulated to meet nutrient requirements according to the Indian Standard 5672 (1992) except Se for the experimental feeding period of 9-20 weeks. Samples of the experimental feed were analyzed for dry matter, crude protein, ether extract, crude fiber, total ash and acid insoluble ashas per AOAC (1995). Calcium and phosphorus was measured according to the method modified by Talapatra *et al*. (1940). The Se content of the feed samples was

Table 1: Formulations of experimental diets

Composition of experimental diets				Proximate composition (percentage dry weight)	
Ingredients	Percentage	Additives	Percentage	Parameters	Dry weight (%)
Maize	56	Biocholine	0.50	Crude protein	17.170
Soyabean meal	21	Biobantox	0.50	Ether extract	2.520
Decoiled rice bran	20	Layvit	0.50	Crude fiber	6.950
Mineral mixture (premix)	2.7	Livoline	0.25	Total ash	10.940
Common salt	0.3	E-sel-powder	0.10	Nitrogen free extract	62.420
L-lysine	0.03	K-zyme	0.50	Calcium	0.920
DL-methionine	0.05			Available phosphorus	0.570
				Metabolisable energy (kcal kg^{-1})	2500.000
				Se (ppm)	0.032

Table 2: Concentration of selenium in experimental diets of different treated groups

Groups	Selenium sources (mg kg^{-1} diet)
T_1	-
T_2	Sodium selenite (0.3)
T_3	Nano-Se (0.075)
T_4	Nano-Se (0.15)
T_5	Nano-Se (0.3)
T_6	Nano-Se (0.6)

estimated by using atomic absorption spectrophotometer. The ingredient composition and proximate composition of the experimental ration is presented in Table 1. The dietary treatments of the experiment group were presented in Table 2.

Growth and feed consumption: Weights of all the individual grower birds in each group were determined at initial and at the end of experiment. At the same time, survival was also determined by counting the individuals in each group. The relative gain rate was calculated using the equation i.e:

$$\frac{\text{Final weight-initial weight}}{\text{Initial weight}}\times 100$$

The Feed Conversion Ratio (FCR) was expressed as:

$$\frac{\text{Total feed consumed-total feed residue}}{\text{Total final weight-total initial weight} + \text{total mortality weight}}$$

Biochemical analysis: Blood and serum samples were collected at 20th weeks of post feeding for biochemical analysis. The serum biochemical indices determined were serum glucose, cholesterol, urea, alkaline phosphate (ALP), aspirate amino transferase (AST), alanine amino transferase (ALT) total protein, albumin, globulin, calcium (Ca) and phosphorus (P) were determined by using Crest biosystems (Goa, India) Kit.

Haematological parameters: Blood and serum samples were collected at 20th weeks of post feeding for haematological studies. The haemoglobin content and Packed Cell Volume (PCV) were

determined as per methods described by Schalm *et al.* (1975) and Jain (1986), respectively. Total Erythrocyte Count (TEC) was estimated using Neubaur's hemocytometer.

Processing of organs: After 20th weeks of post feeding, 15 birds were randomly chosen from each treatment and slaughtered for collection of liver, breast muscles, pancreas, kidney, feathers, spleen and thymus. The birds were kept off fed overnight before bleeding and only water was provided. The live weight of the birds was recorded as pre slaughter weight. The birds were bled by modified Kosher's method (Panda and Mohapatra, 1989). Spleen and thymus were clipped from the viscera with a pair of scissors by holding with a pair of forceps. Spleen and thymus were weighed in a top pan electronic balance. Feather samples were taken from the pectoral and ventral region from the same number of birds per group. Feathers were washed three times with acetone alternating with deionized water.

Selenium deposition in different tissues: The collected liver, breast muscles, pancreas, kidney and feathers samples were oven dried at 100°C for 24 h and finely ground. The Se content in the liver, breast muscles, pancreas, kidney and feathers samples were determined by digesting 0.5 g samples and 1 mL of serum samples at 120°C with 5 mL concentrate HNO_3 for 1 h using KEL plus digestion system. The digested samples were cooled and further digested with 30% H_2O_2 at 200°C. The process continued until the content appeared clear and colorless. The digested samples were filtered into a volumetric flask. The contents of digestion tubes were repeatedly washed with triple distilled water to obtain complete extract of the mineral.

Cellular immunity: At 20th weeks of post feeding, five birds (in duplicate) in each treated groups were injected intradermally in the comb with 100 micro gram of Phytohaemaglutinin-P (PHAP) in 0.1 mL of normal saline to measure the cellular immune response by Cutaneous Basophilic Hypersensitivity (CBH) test (Edelman *et al.*, 1986). The thickness of comb was measured using digital caliper before inoculation and 24 h post inoculation and CBH response was calculated using the equation:

$$\text{CBH response} = \frac{\text{Post injection skin thickness}}{\text{Pre} - \text{injection thickness}} \times 100$$

Humoral immunity: The measure of humoral immunity was carried out as per the method described by Abdallah *et al.* (2009). Sheep Red Blood Cells (SRBCs) were used as test antigens to quantitatively analyze specific antibody response as a measure of humoral immunity. At 20th week, birds from each groups (in triplicate) were immunized intravenously via., a wing vein with 0.07 mL packed RBC mixed with 0.93 mL physiological saline (0.9% NaCl) for measurement of primary response. The SRBC were obtained in heparin solution from local sheep (reared at Instructional Livestock Farm, Bhubaneswar, Odisha) and washed three times in physiological saline. Seven days following the antigen challenge, blood samples were collected and serum samples were used to measure humoral immunity. Antibody production to SRBC was measured using haemagglutination technique with microtitre plate U shape of 96 wells according to Bachman and Mashaly (1986) and Kai *et al.* (1988). All the SRBC antibody titers were expressed as $\log_2$ of the reciprocal of the highest serum dilution causing agglutination of SRBCs.

Preparation of erythrocyte pellet: The 5 mL of whole blood was collected into sterilized micro-centrifuge tube containing 0.75 mL of Acid Citrate Dextrose (ACD; citric acid 8.0 g: Sodium citrate 22.0 g and dextrose 25.0 g and volume made to 1 L in distilled water) as anticoagulant. The blood samples were centrifuged at 3000 x g for 10 min at 4°C, plasma and buffy coats were separated. The resulting erythrocyte pellet was washed thrice with Phosphate Buffer Saline (PBS, pH7.4). For the estimation of catalase, glutathione peroxidase (GSH-Px) and SOD, 1 mL of the 1:1 diluted RBCs in PBS were mixed with 9 mL distilled water to prepare a haemolysate of 1:20 dilution.

Estimation of antioxidant enzymes: Different antioxidant enzymatic activities such as Glutathione peroxidase (GSH-Px) activity was assayed by the method of Paglia and Valentine (1967); Super Oxide Dismutase (SOD) activity of RBCs was measured using NBT assays by Masayasu and Hiroshi (1979) and catalase was assayed in erythrocytes by the method of Bergmayer (1983).

Statistical analysis: The Statistical Analysis System (SAS) software (version 6.12) was used to analyze the data.

RESULTS

Body weight and FCR: The growth performance and feed utilization of grower birds supplemented with different Se sources are presented in Table 3. The initial body weights of the experimental birds were significantly different. This experiment was continued in two phases: Starter phase (0-8 weeks) and grower phase (9-20 weeks), the body weight that achieved at the end of starter phase is reported as the initial body weight at grower phase. The final weight at 20th week of all the treated groups and the control group differed significantly ($p<0.05$) with highest body weight was recorded in T_4 group. The best FCR of 4.73 was shown by treatment T_4 group which showed no significant difference with T_5. The FCR of other groups recorded significantly higher than that of T_4 and T_5 groups.

Serum biochemical parameters: Serum biochemical parameters viz., glucose, cholesterol, triglycerides, total protein, albumin, globulin, urea, SGPT, SGOT, ALP, calcium and phosphorus at twenty weeks of age of layer grower birds are presented in the Table 4. Serum glucose, total protein and SGOT levels were increased significantly ($p<0.05$) starting from control (T_1) to T_2, T_3, T_4, T_5 and T_6 groups, whereas, significantly ($p<0.05$) lower serum cholesterol, A/G ratio and SGPT in T_6 groups than that of control group.

Table 3: Growth performance and feed utilization of grower birds supplemented with different Se sources (Nano-Se and sodium selenite of different concentration) and without Se (control)

	Treatment groups					
Parameters	T_1	T_2	T_3	T_4	T_5	T_6
Initial weight (g)	565.33±6.97^c	559.0±4.80^c	596.00±2.5600^b	634.66±6.51^a	593.41±8.3000^b	572.83±7.270^c
Final wight (g) after 20th weeks of post feeding	1208.00±6.64^e	1310.5±8.40^d	1421.58±7.2000^c	1515.41±7.17^a	1486.41±10.910^b	1298.83±14.91^d
FCR	6.49±0.08^a	5.52±0.3^c	5.225±0.045^d	4.73±0.08^e	4.685±0.095^e	6.04±0.080^b

Results were presented as Means±SE of triplicate observations. Means in the same row with different letters were significantly different ($p<0.05$)

Table 4: Serum biochemical profile of grower birds supplemented with different dietary treatments

	Treatment groups					
Parameters	T_1	T_2	T_3	T_4	T_5	T_6
Glucose (mg dL^{-1})	195.15±3.39^{e}	202.045±0.925^{c}	202.21±4^{c}	203.66±2.66^{c}	215.2±1.1^{b}	224.715±2.60^{a}
Cholesterol (mg dL^{-1})	132.99±1.545^{a}	123.03±1.49^{b}	120.62±2.95^{b}	110.505±1.945^{c}	110.865±2.525^{c}	110.865±2.095^{c}
Triglycerides (mg dL^{-1})	37.54±1.42^{a}	35.5±2.05^{a}	34.08±2.19^{a}	34.11±1.56^{a}	32.45±0.78^{a}	33.19±0.54^{a}
Total protein (g dL^{-1})	3.14±0.07^{c}	3.215±0.065^{c}	3.615±0.055^{b}	3.69±0.02^{b}	3.72±0.05^{ab}	3.875±0.005^{a}
Albumin (g dL^{-1})	2.23±0.09^{a}	2.17±0.06^{a}	2.21±0^{a}	2.245±0.105^{a}	2.34±0.02^{a}	2.35±0.05^{a}
Globulin (g dL^{-1})	1±0.07^{b}	0.985±0.065^{b}	1.405±0.055^{a}	1.55±0.02^{a}	1.4±0.05^{a}	1.575±0.005^{a}
A/G ratio	2.23±0.07^{a}	2.205±0.085^{a}	1.57±0.06^{b}	1.445±0.085^{b}	1.665±0.045^{b}	1.485±0.35^{b}
Urea (mg%)	3.76±0.11^{a}	4.05±0.07^{a}	4.125±0.195^{a}	4.22±0.12^{a}	4.215±0.345^{a}	4.415±0.455^{a}
SGPT (U L^{-1})	12.685±0.545^{a}	10.865±0.005^{b}	9.855±0.105^{c}	8.975±0.045^{cd}	8.835±0.075^{d}	9.08±0.15^{d}
SGOT (U L^{-1})	108.55±1.77^{b}	115.66±3.1^{b}	120.375±0.945^{a}	120.56±2.88^{a}	122.505±3.275^{a}	122.85±2.54^{a}
ALP (U L^{-1})	95.775±2.345^{a}	100.725±5.505^{a}	102.225±3.115^{a}	97.85±0.4^{a}	97.295±0.915^{a}	94.485±3.285^{a}
Ca (mg dL^{-1})	9.925±0.175^{b}	9.74±0.09^{b}	10.04±0.19^{b}	10.85±0.42^{a}	10.135±0.205^{ab}	9.815±0.035^{b}
P (mg dL^{-1})	4.215±0.345^{a}	3.835±0.385^{a}	4.175±0.055^{a}	4.035±0.075^{a}	3.96±0.07^{a}	4.115±0.095^{a}

Values bearing different superscripts in a row differ significantly ($p<0.05$)

Table 5: Immunity status of grower birds under different dietary treatments

	Treatment groups					
Parameters	T_1	T_2	T_3	T_4	T_5	T_6
SRBC response	2.59±0.27^{c}	3.25±0.625^{bc}	4.3±0.35^{ab}	4.71±0.435^{ab}	5.31±0.535^{a}	4.59±0.270^{ab}
CBH response	150.10±6.64^{d}	161.25±2.03^{cd}	172.01±3.665^{bc}	191.63±7.740^{a}	181.68±5.165^{ab}	168.09±2.235^{bcd}

Values bearing different superscripts in a row differ significantly ($p<0.05$)

Table 6: Lymphoid organs (% of live weight) of grower birds in different treated groups

	Treatment groups					
Organs (%)	T_1	T_2	T_3	T_4	T_5	T_6
Spleen	0.125±0.005^{a}	0.125±0.005^{a}	0.130±0.01^{a}	0.130±0.01^{a}	0.140±0.01^{a}	0.115±0.005^{a}
Liver	2.165±0.005^{a}	2.165±0.015^{a}	2.175±0.025^{a}	2.205±0.025^{a}	2.145±0.015^{a}	2.120±0.01^{a}
Thymus	0.150±0.001^{a}	0.153±0.002^{a}	0.147±0.0005^{a}	0.155±0.0025^{a}	0.153±0.002^{a}	0.156±0.0005^{a}

Values bearing different superscripts in a row differ significantly ($p<0.05$)

Immunity status: Antibody titer against SRBC and CBH response (Table 5) and weight of lymphoid organs (Table 6) were used as measures to study the immunity status of the layer grower birds. The antibody titers (log_2) against SRBC immunization of 20th week grower birds were significantly higher ($p<0.05$) in Nano-Se supplemented groups as compared to control (T_1) group. The CBH response was found to be significantly higher ($p<0.05$) in T_4 and T_5 groups. The average weights of lymphoid organs viz., spleen and thymus, expressed as percentage of live body weight at 20th week, showed no significant ($p>0.05$) difference among different treated groups.

Antioxidant enzyme activities: The antioxidant enzyme activities of grower birds are presented in Table 7. Erythrocyte catalase activity was significantly ($p<0.05$) higher in T_2, T_3, T_4, T_5 and T_6 as compared to control (T_1) group and highest level was observed in T_6. Glutathione peroxidase (GPX) activities were significantly ($p<0.05$) highest in T_6. Nano-Se supplemented groups showed

Table 7: Antioxidant enzyme activities in different treated groups supplemented with different selenium sources of grower birds

	Treatment groups					
Parameters	T_1	T_2	T_3	T_4	T_5	T_6
Catalase (moles of H_2O_2 /moles of Heme/min)	213±5.0^{d}	279.91±2.68^{c}	326.15±6.065^{c}	367.91±10.38^{b}	351.89±10.39^{b}	381.19±10.69^{a}
GPX (moles of NADPH2/moles of Heme/min)	2013.94±11.38^{d}	2193.255±60.57^{d}	4622.495±135.655^{c}	6407.83±174.98^{b}	6756.28±129^{ab}	7084.25±151.33^{a}
SOD (U moles^{-1} of Heme)	7.35±0.15^{e}	11.695±0.835^{e}	26.055±1.825^{d}	67.665±2.345^{a}	41.435±1.415^{c}	57.22±1.63^{b}

Values bearing different superscripts in a row differ significantly ($p<0.05$)

Table 8: Effect of different dietary selenium sources and levels on different haematological parameters of grower birds

	Treatment groups					
Parameters	T_1	T_2	T_3	T_4	T_5	T_6
Hb (%)	10.10±1.4^{a}	10.95±1.35^{a}	11.30±1.2^{a}	11±0.5^{a}	10.9±1.1^{a}	10.1±0.9^{a}
TEC (millions/cubic mm)	1.95±0.15^{a}	2.15±0.15^{a}	2.25±0.25^{a}	2.25±0.25^{a}	2.4±0.1^{a}	2.0±0.1^{a}
PCV (%)	27.55±0.95^{a}	29.70±0.9^{a}	30.55±0.05^{a}	30.65±0.15^{a}	30.8±0^{a}	29.85±0.95^{a}

Mean values within a row with different superscripts differ significantly ($p<0.05$)

Table 9: Effect of different dietary selenium sources and level on selenium concentration in serum and tissues of grower birds

	Treatments groups					
Concentration of se	T_1	T_2	T_3	T_4	T_5	T_6
Serum (μg mL^{-1})	0.160±0.002^{f}	0.1755±0.0025^{e}	0.1935±0.0015^{d}	0.2195±0.0035^{c}	0.2435±0.0015^{b}	0.2615±0.0035^{a}
Liver (μg g^{-1})	0.343±0.002^{f}	0.5065±0.0055^{e}	0.5585±0.0065^{d}	0.6160±0.001^{c}	0.7215±0.0035^{b}	0.7670±0.002^{a}
Breast muscle (μg g^{-1})	0.135±0.003^{f}	0.1545±0.0015^{e}	0.2115±0.0035^{d}	0.2700±0.002^{c}	0.3050±0.007^{b}	0.3280±0.003^{a}
Pancreas (μg g^{-1})	0.2115±0.0065^{f}	0.2505±0.0055^{e}	0.31650±0.0045^{d}	0.4480±0.006^{c}	0.5820±0.004^{b}	0.6200±0.008^{a}
Kidney (μg g^{-1})	0.3185±0.0065^{e}	0.4490±0.063^{d}	0.51850±0.0065^{d}	0.7080±0.007^{c}	0.8135±0.0115^{b}	0.8820±0.003^{a}
Feathers (μg g^{-1})	0.2560±0.003^{f}	0.3305±0.016^{e}	0.3945±0.055^{d}	0.4545±0.0055^{c}	0.4915±0.0065^{b}	0.5385±0.0035^{a}

Mean values within a row with different superscripts differ significantly ($p<0.05$)

better GPX activity than sodium selenite and control groups. Similarly, Super Oxide Dismutase (SOD) activity were significantly ($p<0.05$) higher in T_6 group as compared to other treated groups.

Hematological parameters: Different hematological parameters of different treated groups are presented in Table 8. The haemoglobin content, TEC and PCV values showed no significant ($p>0.05$) difference among different treated groups.

Bioavailability of selenium: The bioavailability of Se in different tissues of layer grower birds in different dietary treated groups is presented in Table 9. The Se levels in serum, liver, breast muscle, pancreas, kidney and feathers were significantly higher (in increasing order with respect to increasing selenium concentration in the diet) in all the Nano-Se treated groups (T_3, T_4, T_5 and T_6) than the untreated control (T_1) and sodium selenite treated group (T_2).

DISCUSSION

The present study showed that the growth performance of grower birds was affected by dietary Se level. The improvement in the live body weight of birds fed selenium could be attributed to some

of its biological function such as its role on enzymatic oxidation-reduction, nucleic acid metabolism and in promoting the activity of easily oxidized substances as carotenoides and vitamin A. In addition, improved feather growth may be due to improved growth performance (BWG) or may be due to increase of protein and water in cells (Tayeb and Quader, 2012). In contrast to current results, some reports demonstrated no effect of Se source or Se level on daily gain, feed intake or gain: feed ratio (Payne and Southern, 2005; Yoon *et al*., 2007). The differences were possibly due to the background Se in the feedstuffs. Ryu *et al*. (2005) also reported that feeding even higher concentrations (1-8 ppm) of dietary Se from an inorganic source did not affect the BW of broilers. However, in the present study, increased growth performance was observed when 0.6 mg kg^{-1} of supplemental Nano-Se was fed. This suggests that the addition of 0.6 mg kg^{-1} of Nano-Se was acceptable in avian feeding. The results indicated that the range between optimal and toxic dietary levels of Nano-Se was wider than that of sodium selenite. Improved FCR of broilers fed organic Se supplemented diet could be related to the increased concentrations of the active form of thyroid hormone in the serum of chickens supplemented with Se as well as to the immunomodulating properties of Se (Zelenka and Fajmonova, 2005; Surai, 2006; Hoffmann, 2007; Ozkan *et al*., 2007).

Serum glucose, total protein and SGOT levels were increased significantly ($p<0.05$) starting from control (T_1) to T_2, T_3, T_4, T_5 and T_6 groups. Whereas, significantly ($p<0.05$) lower serum cholesterol, A/G ratio and SGPT in T_6 groups than that of control group were observed. Similarly, the findings of Yang *et al*. (2012) revealed that the aspartate amino transferase, alkaline phosphatase (ALP), globulin, total bilirubin, urea and high density lipoprotein levels were observed to be non-significant between control and Se supplemented group in chicks. The rise in blood glucose may indicate disrupted carbohydrate metabolism due to enhanced breakdown of liver glycogen, possibly mediated by an increase in adrenocorticotrophic and glucagon hormones and/or reduced insulin activity (Raja *et al*., 1992).

In the present study, dietary supplementation of Nano-Se increased both humoral and cellular immunity as measured by antibody titer against SRBC, CBH responses and gave better antibody response than that of sodium selenite and untreated control groups. An increased antibody titre against SRBCs was found when selenium and vitamin E were included at higher levels in the diet (Marsh *et al*., 1981; Gore and Qureshi, 1997; Larsen *et al*., 1997; Leshchinsky and Klasing, 2001; Nageswara *et al*., 2003). On the contrary, Friedman *et al*. (1998) observed depression in antibody production against *E. coli* and Newcastle disease virus vaccination in both chicken and turkey when vitamin E supplementation in the diet was increased from 10 to 150 mg kg^{-1}. Selenium supplementation might have influenced intracellular transmission of signals necessary to initiate proliferation of lymphocytes (Schumacher *et al*., 1990) leading to increase in the antibody titre.

The results of this study also showed higher GSH-Px activity, SOD and catalase activities in erythrocytes of grower birds as compared to the sodium selenite and untreated control groups. Increased glutathione peroxidase activity was found in groups fed Nano-Se compared with the control group in serum and tissue. Feeding Nano-Se could improve the glutathione peroxidase activities and the elevation of the glutathione peroxidase activities in serum, liver and muscle may be optimized with the supplementation of 0.3 mg kg^{-1} of Nano-Se (Cai *et al*., 2012). The differences in GSH-Px activity due to Se source are in agreement with the results presented by Cantor *et al*. (1982), Hassan *et al*. (1988) and Spears *et al*. (2003), who reported that Se supplementation increased plasma GPx3 activity.

The present finding stated that the Nano-Se had higher Se retention in liver, pancreas and breast muscle and was consistent with previously published results in goats (Shi *et al*., 2011). There

are several reports of Se supplementation increasing breast, liver or plasma Se levels (Scott and Thompson, 1971; Cantor *et al.*, 1982; Echevarria *et al.*, 1988a, b; Downs *et al.*, 2000; Spears *et al.*, 2003). Furthermore, Cantor *et al.* (1982) and Spears *et al.* (2003) both indicated that organic Se increased tissue Se levels more than inorganic Se or a diet with no supplemental Se. Choct *et al.* (2004) found that an increasing supplementation rate of Se from 0.1-0.25 mg kg^{-1} increased the breast muscle selenium concentration from 0.232-0.278 mg kg^{-1} and both selenium source (organic and inorganic) and concentration significantly influenced ($p \leq 0.05$) the selenium content of the excreta at day 28. Se supplementation increased plasma Se levels in either turkey poultry's or broilers (Cantor *et al.*, 1975a, b; Cantor *et al.*, 1982; Echevarria *et al.*, 1988b; Spears *et al.*, 2003). The results indicated that Nano-Se was more accumulated in muscle than that of sodium selenite. Elemental selenium nanoparticles have higher bioavailability compared to other selenium compounds, as it was proven in broiler chicken and goat compared with selenite (Shi *et al.*, 2011; Zhou and Wang, 2011). These differences may be the result of the differences in lipophylic properties and metabolic pathways of the species (Zhang *et al.*, 2008). The higher retention of Nano-Se in muscle may effectively reduce the available Se for inducing the selenosis response (Kim and Mahan, 2001). This may be partly the reason for the lower toxicity of Nano-Se compared with selenite. The different retention of Nano-Se and sodium selenite was probably related to the different absorption process and metabolic pathways. It has been reported that nanoparticle show new characteristics of transport and uptake and exhibit higher absorption efficiencies (Davda and Labhasetwar, 2002; Chithrani and Chan, 2007; Zha *et al.*, 2008; 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 and improved intestinal absorption due to the formation of nanoemulsion droplets.

CONCLUSION

The present study showed that dietary supplementation of Nano-Se improved the body weight, feed consumption ratio, antioxidant status, immunity and tissue Se concentrations of grower birds. Considering improvements in all the parameters studied, 0.3 mg Nano-Se per kg (on dry matter basis) of grower ratio is optimum. Further studies related to absorptive mechanism, metabolic pathway, ideal dose required for commercial poultry farming should be needed.

ACKNOWLEDGEMENTS

Authors are highly thankful to (1) National Fellow project on "Nanotechnology in aquaculture: An alternative approach for fish health management and water remediation", ICAR, New Delhi, (2) All India coordinated research project on "Improvement of feed resources and nutrient utilization in raising animal production" of ICAR, New Delhi and (3) INSPIRE Fellowship under INSPIRE Program of the Department of Science and Technology (DST), New Delhi for providing all financial support and other facilities to carry out this study.

REFERENCES

AOAC, 1995. Official Methods of Analysis. 13th Edn., Association of Official Analytical Chemists, Washington, DC.

Abdallah, A.G., O.M. El-Husseiny and K.O. Abdel-Latif, 2009. Influence of some dietary organic mineral supplementations on broiler performance. Int. J. Poult. Sci., 8: 291-298.

Bachman, S.E. and M.M. Mashaly, 1986. Relationship between circulating thyroid hormones and humoral immunity in immature male chickens. Dev. Comp. Immunol., 10: 395-403.

Bergmayer, H.U., 1983. UV Method of Catalase Assay. In: Methods of Enzymatic Analysis, Volume 2: Samples, Reagents, Assessment of Results, Bergmayer, H.U. (Ed.). 3rd Edn., Verlag Chemie, Weinheim, Germany, ISBN-13: 978-0895732323,.

Cai, S.J., C.X. Wu, L.M. Gong, T. Song, H. Wu and L.Y. Zhang, 2012. Effects of Nano-Selenium on performance, meat quality, immune function, oxidation resistance and tissue selenium content in broilers. Poult. Sci., 91: 2532-2539.

Cantor, A.H., M.L. Scott and T. Noguchi, 1975a. Biological availability of selenium in feedstuffs and selenium compounds for prevention of exudative diathesis in chicks. J. Nutr., 105: 96-105.

Cantor, A.H., M.L. Langevin, T. Noguchi and M.L. Scott, 1975b. Efficacy of selenium in selenium compounds and feedstuffs for prevention of pancreatic fibrosis in chicks. J. Nutr., 105: 106-111.

Cantor, A.H., P.D. Moorhead and M.A. Musser, 1982. Comparative effects of sodium selenite and selenomethionine upon nutritional muscular dystrophy, selenium-dependent glutathione peroxidase and tissue selenium concentrations of turkey poults. Poult. Sci., 61: 478-484.

Chithrani, B.D. and W.C. Chan, 2007. Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. Nano Lett., 7: 1542-1550.

Choct, M., A.J. Naylor and N. Reinke, 2004. Selenium supplementation affects broiler growth performance, meat yield and feather coverage. Br. Poult. Sci., 45: 677-683.

Davda, J. and V. Labhasetwar, 2002. Characterization of nanoparticle uptake by endothelial cells. Int. J. Pharm., 233: 51-59.

Downs, K.M., J.B. Hess and S.F. Bilgili, 2000. Selenium source effect on broiler carcass characteristics, meat quality and drip loss. J. Applied Anim. Res., 18: 61-72.

Echevarria, M.G., P.R. Henry, C.B. Ammerman, P.V. Rao and R.D. Miles, 1988a. Estimation of the relative bioavailability of inorganic selenium sources for poultry. 1. Effect of time and high dietary selenium on tissue selenium uptake. Poult. Sci., 67: 1295-1301.

Echevarria, M.G., P.R. Henry, C.B. Ammerman, P.V. Rao and R.D. Miles, 1988b. Estimation of the relative bioavailability of inorganic selenium sources for poultry. 2. Tissue uptake of selenium from high dietary selenium concentrations. Poult. Sci., 67: 1585-1592.

Edelman, A.S., P.I. Sanchez, M.E. Robinson, G.M. Hochwald and G.J. Thorbecke, 1986. Primary and secondary wattle swelling response to phytohemagglutinin as a measure of immunocompetence in chickens. Avian Dis., 30: 105-111.

Friedman, A., I. Bartov and D. Sklan, 1998. Humoral immune response impairment following excess vitamin E nutrition in the chick and turkey. Poult. Sci., 77: 956-962.

Gore, A.B. and M.A. Qureshi, 1997. Enhancement of humoral and cellular immunity by vitamin E after embryonic exposure. Poult. Sci., 76: 984-991.

Hassan, S., J. Hakkarainen, P. Lindberg and S. Sankari, 1988. Comparative effect of dietary sodium selenite on whole blood and plasma selenium and glutathione peroxidase in the chick. Nutr. Rep. Int., 38: 865-871.

Hoffmann, P.R., 2007. Mechanisms by which selenium influences immune responses. Arch. Immunol. Ther. Exp., 55: 289-297.

Indian Standard 5672, 1992. Mineral mixtures for supplementing poultry feeds-specification. Bureau of Indian Standards (BIS), New Delhi, India, December 1992.

Jain, M.C., 1986. Veterinary Hematology. 4th Edn., Lea and Febiger, Philadelphia, PA., USA.

Kai, O.H., N. Nagase, M. Ishikawa, K. Suzuki and K. Sato, 1988. Effects propylthiouracial (PTU) on the immunological status of the chickens. Dev. Comp. Immunol., 12: 145-145.

Kim, Y.Y. and D.C. Mahan, 2001. Comparative effects of high dietary levels of organic and inorganic selenium on selenium toxicity of growing-finishing pigs. J. Anim. Sci., 79: 942-948.

Larsen, C.T., F.W. Pierson and W.B. Gross, 1997. Effect of dietary selenium on the response of stressed and unstressed chickens to *Escherichia coli* challenge and antigen. Biol. Trace Elem. Res., 58: 169-176.

Leshchinsky, T.V. and K.C. Klasing, 2001. Relationship between the level of dietary vitamin E and the immune response of broiler chickens. Poult. Sci., 80: 1590-1599.

Liao, C.D., W.L. Hung, K.C. Jan, A.I. Yeh, C.T. Ho and L.S. Hwang, 2010. Nano/sub-microsized lignan glycosides from sesame meal exhibit higher transport and absorption efficiency in Caco-2 cell monolayer. Food Chem., 119: 896-902.

Marsh, J.A., R.R. Dietert and G.F. Combs, 1981. Influence of dietary selenium and vitamin E on the humoral immune response of chicks. Proc. Soc. Exp. Biol. Med., 166: 228-236.

Masayasu, M. and Y. Hiroshi, 1979. A simplified assay method of superoxide dismutase activity for clinical use. Clinica Chimica Acta, 92: 337-342.

Mohapatra, P., R.K. Swain, S.K. Mishra, T. Behera and P. Swain *et al*., 2014. Effect of dietary nano selenium on tissue deposition, antioxidant status and immune functions in layer chicks. Int. J. Pharmacol., 10: 160-167.

Nageswara, A.R., R.V. Nageswara and R.M. Nageswara, 2003. Effect of *E-care-se*-herbal on the performance on immune response in broilers. Indian. J. Pout. Sci., 38: 115-120.

Ozkan, S., H.B. Malayoglu, S. Yalcin, F. Karadas and S. Kocturk *et al*., 2007. Dietary vitamin E (á-tocopherol acetate) and selenium supplementation from different sources: Performance, ascites-related variables and antioxidant status in broilers reared at low and optimum temperatures. Br. Poult. Sci., 48: 580-593.

Paglia, D.E. and W.N. Valentine, 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 70: 158-169.

Panda, B. and S.C. Mohapatra, 1989. Bleeding of Poultry Modified by Kosher's Method, Poultry Production. ICAR Publication, New Delhi, Pages: 121.

Pappas, A.C. and E. Zoidis, 2012. The Role of Selenium in Chicken Physiology: New Insights. In: Chickens: Physiology, Diseases and Farming Practices, Kapur, I. and A. Mehra (Eds.). Nova Science Publishers, New York, USA., ISBN-13: 9781620810279, pp: 167.

Payne, R.L. and L.L. Southern, 2005. Comparison of inorganic and organic selenium sources for broilers. Poult. Sci., 84: 898-902.

Raja, M., A. Al-Fatah, M. Ali, M. Afzal, R.A. Hassan, M. Menon and M.S. Dhami, 1992. Modification of liver and serum enzymes by paraquat treatment in rabbits. Drug Metab. Drug Interact, 10: 279-291.

Ryu, Y.C., M.S. Rhee, K.M. Lee and B.C. Kim, 2005. Effects of different levels of dietary supplemental selenium on performance, lipid oxidation and color stability of broiler chicks. Poult. Sci., 84: 809-815.

Schalm, O.W., N.C. Jain and E.J. Carroll, 1975. Veterinary Haematology. 3rd Edn., Lea and Febiger, Washington Square, Philadelphia, USA., pp: 66-78.

Schumacher, L.K., M. Roy, M.I. Wishe, M.W. Cohenand and G. Stotzky, 1990. Selenium and immune functions. 1. Effect on lymphocyte proliferation and production of interleukin 1 and interleukin 2. Proc. Soc. Exp. Biol. Med., 193: 136-141.

Scott, M.L. and J.N. Thompsom, 1971. Selenium content of feedstuffs and effects of dietary selenium levels upon tissue selenium in chicks and poults. Poult. Sci., 50: 1742-1748.

Shi, L., W. Xun, W. Yue, C. Zhang and Y. Ren *et al.*, 2011. Effect of sodium selenite, Se-yeast and nano-elemental selenium on growth performance, Se concentration and antioxidant status in growing male goats. Small Rumin. Res., 96: 49-52.

Spears, J.W., J. Grimes, K. Lloyd and T.L. Ward, 2003. Efficacy of a novel organic selenium compound (zinc-l-selenomethionine, available Se) in broiler chicks. Proceedings of the 1st Congress of the Latin American Annual Nutrition College, August 18-23, 2003, Cancun, Mexico, pp: 197-198.

Surai, P.F., 2006. Selenium in Nutrition and Health. 1st Edn. Nottingham University Press, Nottingham, UK.

Talapatra, S.K., S.N. Ray and K.C. Sen, 1940. Estimation of phosphorus, chlorine, calcium, magnesium, sodium and potassium in foodstuffs. Indian J. Vet. Sci. Anim. Husb., 10: 243-246.

Tayeb, I.T. and G.K. Qader, 2012. Effect of feed supplementation of selenium and vitamin e on production performance and some hematological parameters of broiler. KSU J. Nat. Sci., 15: 46-56.

Yang, Y.R., F.C. Meng, P. Wang, Y.B. Jiang and Q. Yin *et al.*, 2012. Effect of organic and inorganic selenium supplementation on growth performance, meat quality and antioxidant property of broilers. Afr. J. Biotechnol., 11: 3031-3036.

Yoon, I., T.M. Werner and J.M. Butler, 2007. Effect of source and concentration of selenium on growth performance and selenium retention in broiler chickens. Poult. Sci., 86: 727-730.

Zelenka, J. and E. Fajmonova, 2005. Effect of age on utilization of selenium by chickens. Poult. Sci., 84: 543-546.

Zha, L.Y., Z.R. Xu, M.Q. Wang and L.Y. Gu, 2008. Chromium nanoparticle exhibits higher absorption efficiency than chromium picolinate and chromium chloride in Caco-2 cell monolayers. J. Anim. Physiol. Anim. Nutr., 92: 131-140.

Zhang, J.S., X.F. Wang and T.W. Xu, 2008. Elemental selenium at nano size (Nano-Se) as a potential chemopreventive agent with reduced risk of selenium toxicity: Comparison with Se-methylselenocysteine in mice. Toxicol. Sci., 101: 22-31.

Zhang, J.S., H.L. Wang, X.X. Yan and L. Zhang, 2005. Comparison of short-term toxicity between Nano-Se and selenite in mice. Life Sci., 76: 1099-1109.

Zhang, J.S., X.Y. Gao, L.D. Zhang and Y.P. Bao, 2001. Biological effects of a nano red elemental selenium. Biofactors, 15: 27-38.

Zhou, X. and Y. Wang, 2011. Influence of dietary nano elemental selenium on growth performance, tissue selenium distribution, meat quality and glutathione peroxidase activity in Guangxi Yellow chicken. Poult. Sci., 90: 680-686.