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Effects of Dietary Nano-Selenium on Tissue Selenium Deposition, Antioxidant Status and Immune Functions in Layer Chicks

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Abstract: Comparative study on the effect of nano selenium (nano-Se) and sodium selenite on the growth, bioavailability, antioxidative activities, hematological and biochemical parameters, cellular and humoral immunity was done in layer chicks upto 8th week post feeding. The results showed significant differences ($p<0.05$) in relative weight gain and final body weight of the nano-Se treated groups upto a dose of 0.3 mg kg^{-1} of diet as compared to sodium selenite and control groups. However, further increase in dietary nano-Se content in feed had negative effect on weight and Relative Gain Rate (RGR). Survival rate and Feed Conversion Ratio (FCR) were not affected by dietary treatments. Chicks fed with both nano-Se and sodium selenite showed higher ($p<0.05$) Se content in different tissues (breast muscle, liver, kidney, pancreas, serum and feathers). However, highest value ($p<0.05$) of Se content in breast muscle and liver was observed in nano-Se treated groups. Selenium concentrations in serum, liver and breast muscle increased linearly and quadratically ($p<0.05$) as dietary Se level increased for all Se sources but its magnitude was substantially greater ($p<0.05$) when nano-Se was fed. Glutathione peroxidase (GSH-Px), erythrocyte catalase (CAT) and superoxide dismutase (SOD) activities were significantly different ($p<0.05$) in all treated groups than control. Dietary nano-Se also increased several serum biochemical and haematological parameters. In addition, it significantly increased both cellular and humoral immunity in layer chicks after 8th weeks of post feeding. In conclusion, dietary administration of nano-Se was found superior than that of inorganic sodium selenite in various aspects in layer chicks. Further extensive study for exploring absorption mechanisms, metabolic pathways, ideal dose/form of nano-Se is suggested for optimum utilization of nano-material based application of Se feeding in poultry.

Key words: Nano-selenium, nanotechnology, antioxidant, biochemical parameters, bioavailability, haematology, cellular immunity, humoral immunity, layer chicks, poultry feeding

INTRODUCTION

The rapid development of nano-technology 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. Recently, selenium (Se) has been recognized as an essential dietary nutrient. Dietary selenium is an essential trace element for animals and humans with a variety of biological functions (Surai, 2006). These compounds are necessary for growth, fertility, immune system, hormone metabolism, cell growth and antioxidant defence systems in animals and humans (Pappas and Zoidis, 2012).

Selenium deficiency in poultry, causes some diseases which include exudative diathesis, pancreatic dystrophy and nutritional muscular dystrophy (McDowell, 1992). Selenium is found naturally in plant feed ingredients but concentrations vary greatly depending on both the plant species and Se status of the soil. Therefore, poultry diets require supplementry Se in

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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 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. 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 chicks. Limited studies on nano-Se supplementation are available and the findings are rather inconsistent particularly as regards to several physiological effects in chicks. Thus, the purpose of this experiment was to study effects of dietary nano-Se on growth performance, tissue deposition, antioxidant defense system and immune functions in layer chicks in comparison to sodium selenite.

MATERIALS AND METHODS

Selenium sources: Nano red elemental selenium particles (nano-Se) were synthesized 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.

Animals' diet and experimental procedures: Vaccinated day old sexed 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. The chicks were randomly distributed so as to eliminate any significant difference between treatments with respect to body weight. The chicks were protected against Newcastle and infectious bursal diseases by routine vaccination. The chicks were provided 24 h free access to clean drinking water. From 0-4 weeks of age, artificial light was provided to chicks to achieve brooding temperature and further the day length was the photoperiod for the birds during chick and grower stage.

Basal diet was formulated to meet nutrient requirements according to the National Research Council (1994) except Se for the experimental feeding period of 0-8 weeks. Samples of the experimental feed were analyzed for dry matter, crude protein, ether extract, crude fiber, total ash and acid insoluble ash. Calcium and phosphorus was measured according to the method modified by Talapatra *et al*. (1940). The Se content of the feed samples was 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 chicks in each group were determined at initial

Table 1: Formulations of experimental diets

Formulations of experimental diets				Proximate composition	
Ingredients	%	Additives	%	Parameters	(% dry weight)
Maize	54	Biocholine	0.50	Moisture	88.96
Soyabean meal	32	Biobantox	0.50	Crude protein	20.28
Deoiled rice bran	11	Layvit	0.50	Ether extract	2.33
Mineral mixture (premix)	2.7	Livoline	0.25	Crude fiber	5.17
Common salt	0.3	E-sel-powder	0.10	Total ash	9.06
L-Lysine	0.03	K-zyme	0.50	Nitrogen free extract*	63.16
DL-methionine	0.05			Calcium	1.08
				Available phosphorus	0.65
				Metabolisable energy* (Kcal kg^{-1})	2750.00
				Se (ppm)	0.03

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

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

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.:

$$\text{Final weight } (\%) = \frac{\text{Initial weight}}{\text{Final weight}} \times 100$$

The Feed Conversion Ratio (FCR) was expressed as:

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

Biochemical analysis: Blood and serum samples were collected at 8th 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 8th week 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 8th week of post feeding, 15 birds were randomly chosen from each treatment and slaughtered for collection of liver, breast muscles, pancreas, kidney, feathers, spleen, bursa of fabricius 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 chicks were bled by modified Kosher's method (Panda and Mohapatra, 1989). Spleen, bursa of fabricius and thymus were clipped from the viscera with a pair of scissors by holding with a pair of forceps. Spleen, bursa of fabricius and thymus were weighed in a top pan electronic balance.

Selenium content 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 8th weeks of post feeding, 5 birds (in duplicate) in each treated groups were injected intra dermally in the comb with 100 μg 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:

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

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 measure of humoral immunity. At 8th weeks of post feeding, chicks 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 SRBCs 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 SRBCs was measured using haemagglutination technique with microtitre plate U shape of 96 wells plates according to Bachman and Mashaly (1986) and Kai *et al.* (1988). All SRBCs antibody titers were expressed as $\log_2$ of the reciprocal of the highest serum dilution causing agglutination of SRBCs.

Preparation of erythrocyte pellet: Five milliliter 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×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, pH 7.4). RBC diluted to 1:1 in PBS was used for the estimation of haemoglobin. For the estimation of catalase, SOD, lipid peroxidation (LPO) and glutathione peroxidase (GSH-Px), 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 by the method of Paglia and Valentine (1967) Super Oxide Dismutase (SOD) activity of RBCs were measured using NBT assays by Masayasu and Hiroshi (1979) and catalase was assayed in erythrocytes by the method of Bergmayer (1983).

Statistical analysis: SAS (1991) software (version 6.12) was used to analyze the data.

RESULTS

Body weight and FCR: The weekly average body weight and FCR of layer chicks under different treated groups upto 8th week of post feeding were presented in Table 3. At the beginning upto 3rd week of post feeding, no significant difference was observed in the initial weight between different treated groups and the control. However, there was significant difference ($p<0.05$) in RGR and final weight of all different treated groups (T_1, T_2, T_3, T_4 and T_5) as compared to control group after 8th weeks of post feeding. Moreover, RGR and final weight of T_2, T_3 and T_4 group were significantly increased as compared to T_1 and T_6 group. However, RGR and final weight in T_6 group was higher as compared to T_1. Survival rate and FCR were not affected by the dietary treatments after 8th week of post feeding.

Serum biochemical parameters: Serum biochemical parameters viz., glucose, cholesterol, triglycerides, total protein, albumin, globulin, urea, SGPT, SGOT, ALP, calcium and phosphorus at eight weeks of age of layer chicks are presented in the Table 4. Serum glucose, total protein, globulin, SGOT, Urea levels were increased linearly and quadratically ($p<0.05$) starting from control to T_1, T_2, T_3, T_4 and T_5 groups. Whereas, serum cholesterol, triglyceride, A/G ratio, ALP decreased linearly ($p<0.05$) starting from control to T_1, T_2, T_3, T_4 and T_5 groups. In addition to that, serum albumin, Ca and p-level of the layer chicks showed no significant difference ($p>0.05$) in all the treated groups along with the control group.

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

Table 3: Growth performance and feed utilization of layer chicks supplemented with different Se sources (nano-Se and sodium selenite of different concentration) and without Se (control)

	Groups					
Parameters	Control	T_1	T_2	T_3	T_4	T_5
Initial weight (g)	31.75±0.370	31.58±0.290	31.12±0.310	31.16±0.33	31.12±0.29	31.41±0.4000
Final weight (g) after 8th weeks of post feeding	300.83±4.850	498.7±4.760^a	543.45±3.740^b	544.83±4.33^b	541.29±4.61^b	482.45±3.7700^c
RGR (%)	847.49±20.85^a	1479.16±17.76^b	1646.30±11.74^c	1648.49±14.3^c	1639.36±14.61^c	1435.97±13.177^b
FCR	3.30±0.010^a	3.08±0.040^b	2.88±0.050^b	2.88±0.01^b	2.9±0.0200^b	3.02±0.4500^b

RGR: Relative gain rate, FCR: Feed conversion ratio. Results were presented as Mean±SE of triplicate observations. Means in the same row with different letters were significantly different ($p<0.05$)

Table 4: Serum biochemical profile of layer chicks supplemented with different dietary treatments

	Groups					
Parameters	Control	T_1	T_2	T_3	T_4	T_5
Glucose (mg dL^{-1})	97.420±0.540^{aa}	98.55±2.79^b	115.71±2.95^c	114.660±1.12^c	122.98±1.64^c	125.710±2.26^c
Cholesterol (mg dL^{-1})	195.880±8.900^a	184.36±7.62^b	182.43±1.09^b	170.370±5.52^b	169.76±3.98^b	137.540±7.87^c
Triglycerides (mg dL^{-1})	37.360±1.720^a	36.42±3.45^a	33.89±1.92^b	32.480±1.64^b	31.32±0.405^b	30.530±1.505^b
Total protein (g dL^{-1})	2.710±0.045^a	3.05±0.16^b	3.57±0.055^c	3.610±0.03^c	3.66±0.05^c	3.710±0.07^c
Albumin (g dL^{-1})	2.045±0.035^a	2.05±0.01^a	2.105±0.03^a	2.155±0.06^a	2.25±0.04^a	2.285±0.015^a
Globulin (g dL^{-1})	0.670±0.010^a	1.00±0.17^b	1.27±0.08^c	1.450±0.09^d	1.41±0.09^d	1.420±0.06^d
A/G ratio	3.520±0.105^a	3.85±0.06^a	1.66±0.12^b	1.480±0.14^b	1.60±0.13^b	1.430±0.05^b
Urea (mg%)	3.520±0.105^a	3.85±0.06^b	3.89±0.02^b	3.900±0.08^b	3.95±0.39^b	3.960±0.46^b
SGPT (U L^{-1})	11.850±0.270^a	10.43±0.22^b	9.35±0.045^c	8.680±0.045^d	8.44±0.08^d	8.650±0.06^d
SGOT (U L^{-1})	104.520±1.105^a	108.99±3.10^a	116.70±1.14^b	118.620±3.55^b	118.91±4.14^b	119.440±5.43^b
ALP (U L^{-1})	93.960±01.95^a	95.83±2.21^b	97.06±1.54^b	92.490±3.42^a	92.64±3.67^a	91.610±6.16^a
Ca (mg dL^{-1})	9.670±0.060^a	9.82±0.03^a	9.90±0.02^a	9.840±0.055^a	9.86±0.02^a	9.750±0.055^a
P (mg dL^{-1})	3.680±0.035^a	3.75±0.085^a	3.94±0.02^a	3.870±0.02^a	3.81±0.08^a	3.780±0.015^a

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

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

	Groups					
Response	Control	T_1	T_2	T_3	T_4	T_5
SRBC	0.997±0.15^a	1.451±0.15^b	1.972±0.32^c	1.951±0.15^b	1.951±0.15^b	1.972±0.32^c
CBH	120.620±2.44^a	132.240±5.26^b	143.350±1.01^c	146.090±0.06^c	150.730±2.30^c	143.260±1.89^c

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

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

	Groups					
Organs (%)	Control	T_1	T_2	T_3	T_4	T_5
Spleen	0.150±0.001^a	0.151±0.001^a	0.154±0.004^a	0.154±0.005^a	0.153±0.004^a	0.154±0.001^a
Liver	2.310±0.010^a	2.290±0.650^a	2.280±0.049^a	2.400±0.090^a	2.340±0.030^a	2.290±0.040^a
Bursa	0.264±0.002^a	0.269±0.004^a	0.271±0.003^a	0.264±0.005^a	0.270±0.006^a	0.265±0.007^a
Thymus	0.159±0.001^a	0.161±0.001^a	0.158±0.006^a	0.161±0.008^a	0.161±0.003^a	0.166±0.006^a

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

Table 7: Antioxidant enzyme activities in different treated groups supplemented with different selenium sources of layer chicks

	Groups					
Parameters	T_1	T_2	T_3	T_4	T_5	T_6
Catalase (mol of H_2O_2/mol of Heme min^{-1})	214.00±4.010^a	282.20±3.330^b	328.03±7.7800^c	368.84±10.010^c	353.45±10.960^c	382.45±1.550^c
GPX (mol of NADPH2/mol of Heme min^{-1})	2080.50±55.13^a	2270.71±45.08^b	4706.58±147.26^c	6531.37±148.74^c	6905.75±180.17^c	7236.12±150.2^c
SOD (U mol^{-1} of Heme)	8.15±0.350^a	13.04±0.810^b	27.37±1.5200^c	70.31±1.9600^d	64.05±2.8000^d	60.84±2.010^d

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 layer chicks

	Groups					
Parameters	Control	T_1	T_2	T_3	T_4	T_5
Hb (%)	9.55±1.05^a	10.80±1.00^b	10.30±1.10^b	10.64±0.15^b	10.45±0.85^b	9.45±1.05^b
TEC (millions mm^{-3})	1.90±0.10^a	2.10±0.02^b	2.25±0.25^b	2.20±0.30^b	2.15±0.15^b	1.90±0.10^b
PVC (%)	26.50±1.30^a	28.75±1.45^b	29.55±1.05^b	29.25±1.25^b	29.15±1.65^b	26.90±1.40^b

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

status of the layer chicks. The antibody titers (log_2) against SRBCs immunization of 8th week chicks were significantly higher ($p<0.05$) in T_2, T_3, T_4 and T_5 groups as compared to T_1 and control group. The CBH response was found to be significantly higher ($p<0.05$) in T_2, T_3, T_4 and T_5 groups as compared to T_1 and control group. However, T_1 group showed higher antibody response as compared to control against both SRBCs and CBH immunizations. The average weights of lymphoid organs viz., spleen, bursa and thymus was expressed as percentage of live body weight after 8th week of post feeding, showed no significant ($p>0.05$) difference among different treated groups.

Antioxidant enzyme activities: The antioxidant enzyme activities of layer chicks were presented in Table 7. Erythrocyte catalase activity were significantly ($p<0.05$) higher in T_3, T_4, T_5 and T_6 as compared to T_1 and control group. Glutathione peroxidase (GPX) activities were significantly ($p<0.05$) higher in T_3, T_4, T_5 and T_6 as compared to T_1 and control. However, both GPX and catalase activities were significantly higher in T_1 as compared to untreated control group. Similarly super oxide dismutase activity were significantly ($p<0.05$) higher in T_4, T_5 and T_6 group as compared to other treated and control groups.

Hematological parameters: Different hematological parameters of different treated groups are presented in Table 8. The haemoglobin content, TEC and PVC values were significantly ($p>0.05$) higher in all the treated groups (T_1, T_2, T_3, T_4 and T_5) than the control group. However, there is no significance difference among different treated groups.

Bioavailability of selenium: The bioavailability of Se in different tissues of layer chicks 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_2, T_3, T_4 and T_5) than the untreated control and sodium selenite treated group (T_1). However, T1 group showed significantly higher selenium deposition in liver, pancreas and kidney than the untreated control group.

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

	Treatments					
Concentration of Se	Control	T_1	T_2	T_3	T_4	T_5
Serum (μg mL^{-1})	0.152±0.001^a	0.167±0.001^a	0.186±0.0010^a	0.216±0.003^b	0.240±0.002^b	0.252±0.006^b
Liver (μg g^{-1})	0.329±0.004^a	0.490±0.004^b	0.551±0.0135^c	0.608±0.006^d	0.792±0.006^e	0.812±0.008^e
Breast muscle (μg g^{-1})	0.120±0.002^a	0.149±0.001^a	0.202±0.0030^b	0.257±0.004^b	0.292±0.009^b	0.313±0.011^b
Pancreas (μg g^{-1})	0.144±0.021^a	0.208±0.003^b	0.279±0.0040^b	0.316±0.005^c	0.438±0.006^d	0.567±0.011^e
Kidney (μg g^{-1})	0.279±0.006^a	0.334±0.009^b	0.480±0.0080^c	0.616±0.013^e	0.764±0.013^d	0.844±0.021^e
Feathers (μg g^{-1})	0.213±0.002^a	0.269±0.016^a	0.289±0.0040^a	0.312±0.010^b	0.470±0.014^c	0.506±0.004^d

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

DISCUSSION

The supplementation of feed with selenium is usually limited to selenides such as sodium selenite and selenium containing organic compounds. It was found that nano-Se had similar or higher bioavailability and much less toxicity in mice, rat, broiler and goat compared with selenite (Zhang *et al*., 2001, 2005; Gao *et al*., 2002; Jia *et al*., 2005; Wang *et al*., 2007; Shi *et al*., 2011; Zhou and Wang, 2011). The present study showed that the growth performance of chicks was affected after 3rd weeks of post feeding by dietary Se level. 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 of Se in the feedstuffs. A concentration of 0.15 mg Se/kg diet is recommended for broiler chickens (National Research Council, 1994). However, the basal diet used in this experiment contained only 0.075 mg Se/kg diet which was far lower than the requirements. Poultry diets deficient in selenium resulted in poor growth and development, increased mortality and reduced egg production, decreased hatchability (Kim and Mahan, 2003). The present result proved this point and the group not supplemented with any forms of selenium showed the symptoms of selenium deficiency such as lower weight gain and RGR values. On the other hand, dietary Se levels exceeding 0.5 mg kg^{-1} might impair the growth while clinical symptoms of Se toxicity appear above 3-5 mg Se/g of diet (Kirchgessner *et al*., 1997). 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.

It was obvious that the Se contents in layer chick tissues such as breast muscle, liver, kidney, pancreas and feathers were markedly increased with the addition of dietary nano-Se in treated groups than the sodium selenite and untreated control groups. Moreover, increased selenium deposition was found with increasing nano-Se content in the diet. In general, animal study trials demonstrated that bioavailability of nano-Se was higher than that of inorganic forms (sodium selenite) (Zhang *et al*., 2001). Thus, Se bioavailability depended not only on its absorption by the intestine but also on its conversion to a biologically active form (Foster and Sumar, 1995). There are several reports of Se supplementation to increase the breast, liver, or plasma Se levels (Downs *et al*., 2000; Spears *et al*., 2003).

Selenium has a number of biological functions in animals and the most important action is its antioxidant effect (Levander and Burk, 1994). The results of present study also showed higher GSH-Px activity, SOD and catalase activities in erythrocytes of layered chicks as compared to the sodium selenite and untreated control groups. This suggested that serum GSH-Px activity seemed to be reflective of its dietary Se level but additional dietary Se did not stimulated further activity of the enzyme. The present finding indicated 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). Increasing the Se content of food for human consumption by manipulating source and level of Se supplementation to livestock has been area of interest to food scientists (Zhan *et al*., 2007; Wang and Xu, 2008). The results indicated that nano-Se was more greatly accumulated in breast muscle than the sodium selenite.

An improved antioxidant system of the chick may also enhance immune system function which is extremely important at this point in physiological development. 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 higher antibody response than that of sodium selenite and untreated control groups. However, different dietary selenium sources did not affect weight of different lymphoid organs and different haematological parameters such as haemoglobin content, TEC and PVC values. In addition to that, different physiological parameters such as serum glucose, total protein, globulin, SGOT, urea levels were increased linearly and quadratically ($p<0.05$)

with increase in concentration of nano-Se in diet. However, there was no effect on serum cholesterol, triglyceride, A/G ratio, ALP due to dietary administrations of nano-Se in layered chicks. Similarly, the findings of Yang *et al.* (2012) revealed that the aspartate amino transferase, alkaline phosphatase (ALP), total protein, globulin, total bilirubin, glucose, urea, total cholesterol, triglyceride and high density lipoprotein levels were observed to be non-significant between control and Se supplemented group in chicks.

The different physiological effects of nano-Se and sodium selenite were 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 (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.

CONCLUSION

The present study had demonstrated that dietary administration of nano-Se could improve the final weight, relative gain rate, antioxidant status (GSH-Px activities, SOD and erythrocyte catalase activities) and selenium deposition in several tissues especially breast muscle of layer chicks. Moreover, nano-Se appeared to be more effective ($p<0.05$) for than that of inorganic sodium selenite in increasing different biochemical parameters, haematological parameters, cellular and humoral immunity. The results also showed that the range of optimum dietary levels of nano-Se was wider than that of sodium selenite and nano-Se of 0.3 mg kg^{-1} of dry diet is the optimum dose for getting better physiological effects in layer chicks. Further supportive study is needed to explore absorption mechanism, metabolic pathways, ideal dose and form of nano-Se that should be fed to poultry under commercial conditions.

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REFERENCES

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,.

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.

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.

Foster, L.H. and S. Sumar, 1995. Selenium in the environment, food and health. Nutr. Food Sci., 5: 17-23.

Gao, X., J. Zhang and L. Zhang, 2002. Hollow sphere selenium nanoparticles: Their *in-vitro* anti hydroxyl radical effect. Adv. Mater., 14: 290-293.

Jain, N.C., 1986. Schalm's Veterinary Hematology. 4th Edn., Lea and Febigir, Philadelphia, PA., USA., Pages: 600.

Jia, X., N. Li and J. Chen, 2005. A subchronic toxicity study of elemental Nano-Se in Sprague-Dawley rats. Life Sci., 76: 1989-2003.

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

Kim, Y.Y. and D.C. Mahan, 2003. Biological aspects of selenium in farm animals. Asian-Aust. J. Anim. Sci., 16: 435-444.

Kirchgessner, M., S. Gabler and W. Windisch, 1997. Homeostatic adjustments of selenium metabolism and tissue selenium to widely varying selenium supply in ^{75}Se labeled rats. J. Anim. Physiol. Anim. Nutr., 78: 20-30.

Levander, O.A. and R.F. Burk, 1994. Selenium. In: Modern Nutrition in Health and Disease, Shils, M.E., J.A. Olson and M. Shike (Eds.). Lea and Febiger, Philadelphia.

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.

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

McDowell, L.R., 1992. Minerals in Animal and Human Nutrition. 2nd Edn., Academic Press Inc., San Diego, CA., USA., ISBN-13: 9780444513670, pp: 284-332.

National Research Council, 1994. Nutrient Requirements of Poultry. 9th Rev. Edn., National Academy Press, Washington, DC., USA., ISBN-10: 0-309-04892-3, Pages: 176.

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 Inc., New York, USA., ISBN-13: 9781620810279, pp: 51-69.

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

SAS., 1991. SASR System for Regression. 2nd Edn., SAS Institute Inc., Cary, NC., Pages: 210.

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.

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 Latin American Congress of Animal Nutrition, (LACAN'03), 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.

Wang, H.L., J.S. Zhang and H.Q. Yu, 2007. Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: Comparison with selenomethionine in mice. Free Radic. Biol. Med., 42: 1524-1533.

Wang, Y.B. and B.H. Xu, 2008. Effect of different selenium source (sodium selenite and selenium yeast) on broiler chickens. Anim. Feed Sci. Tech., 144: 306-314.

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.

Zhan, X.A., M. Wang, R.Q. Zhao, W.F. Li and Z.R. Xu, 2007. Effects of different selenium source on selenium distribution, loin quality and antioxidant status in finishing pigs. Anim. Feed Sci. Technol., 132: 202-211.

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.

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.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.

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.