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**COMPARATIVE EFFECTS OF NANO, MINERAL AND ORGANIC SELENIUM ON
GROWTH PERFORMANCE, IMMUNITY RESPONSES AND TOTAL ANTIOXIDANT
ACTIVITY IN BROILER CHICKENS**

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ABSTRACT

This study was performed to comparison the effects of Nano elemental selenium (Nano-Se) supplementation with two other sources of Se on growth performance, some immunity responses and total antioxidant activity in Ross male broiler chickens. A 2³ factorial arrangement with 2 level of dietary Se from Nano-Se, mineral-Se or Organic-Se added to a maize–soybean meal diet was conducted. The results showed that in comparison to control, feed consumption during the whole experimental period at the level of 0.5 mg/kg Nano-Se increased significantly ($P < 0.05$).the use of Nano-Se at the level of 0.5 mg/kg caused in the best feed conversion ratio (FCR). Also the level of 0.5 mg/kg Nano-Se showed the most weight gain in comparison to other treatments at the end of experimental period ($P < 0.05$). The level of 0.5 mg/kg Nano-Se caused in significant decrease of abdominal cavity fat ($P < 0.05$). The highest total antioxidant activity of blood serum happened at the level of 0.5 mg/kg Nano-Se ($P < 0.05$). The level of 0.5 mg/kg Nano-Se caused in significant increase of Antibody against influenza virus and Sheep Red Blood Cell (SRBC) in comparison to control group ($P < 0.05$). The results showed that the use of Nano-

Se caused in improvement of function, immunity and total antioxidant activity of blood serum in broiler chickens.

Keywords: Nano, Mineral and Organic Selenium, Broiler Chickens, Immunity Responses

INTRODUCTION

Selenium is an essential trace element that has a large number of biological functions in human and poultry organisms. It is common practice to supplement broiler diets with Se. The most important and known action is its antioxidant effect because it forms selenocysteine, part of the active center of glutathione peroxidase [3]. This enzyme has antioxidant activity and contributes to the oxidant defense by catalyzing the reduction of hydrogen peroxide and lipid peroxides to less harmful hydroxides [2]. The activity level of this enzyme in the liver or plasma is indicative of the Se supply to the organism. In addition, dietary selenium is essential for the activity of virtually all arms of the immune system. Previous studies have shown that in chickens, growth performance, survival, meat quality, and antioxidant protection level are affected by dietary Se status [3, 15]. Many experimental studies have established that selenomethionine and Se-enriched yeast are the most appropriate forms of Se for use in animal nutritional supplements because of their excellent bioavailability and lower toxicity among various forms of Se [26, 24].

With the recent development of nanotechnology, nano-Se has attracted widespread attention because nanometer particulates exhibit novel characteristics such as a large surface area, high surface activity, high catalytic efficiency, strong adsorbing ability, and low toxicity [23]. It has been reported that nano-Se possesses comparable efficiency to selenite and Se-methylselenocysteine in upregulating seleno-enzymes but with dramatically decreased toxicity [23, 1]. Therefore, the purpose of this study was to evaluate the effect of supplementing nano-Se and two other sources of Se (Mineral and Organic) levels on performance, immune function and oxidation resistance.

MATERIALS AND METHODS

Selenium sources

Nano-Se was synthesized by reducing selenite in an environment containing bovine serum albumin (BSA), which is able to adhere to Se atoms and control the size of their aggregation according to Zhang *et al.* [24]. One milliliter of 25 mM sodium selenite was mixed with 4 mL of 25 mM glutathione peroxidase (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 20–80 nm using a Mastersizer particle size and zeta potential analyzer (Malvern Instruments, Malvern, UK), with the average size being 60 nm. Sodium selenite as mineral-Se and L-selenomethionine as organic-Se were purchased from Tehran reagent Co. Ltd (Tehran, Iran).

Animal and experimental procedures

This experiment was performed to explore the effects of Nano-Se in broiler chicks as compared to sodium Selenite and L-selenomethionine. All procedures were approved by the Institutional Animal Care and Use Committee of Islamic Azad University.

The effects of dietary Se source and level on growth performance, Feed conversion ratio and carcass component

A total of 336 Ross male broiler chickens, 1 d of age, were allotted to a completely randomized design in a 2³ factorial arrangement. Chicks were fed diets containing sodium selenite (SS) (0.2 and 0.5 mg/kg), L-selenomethionine (L-Se-Me) (0.2 and 0.5 mg/kg) or Nano-Selenium (Nano-Se) (0.2 and 0.5 mg/kg). The basal diet used in this experiment was Se deficient and contained approximately 0.031 ± 0.002 mg/kg of total Se, as determined by atomic absorption spectrophotometry (model AA6501, Shimadzu Ltd., Kyoto, Japan). Basal diet was formulated to meet nutrient requirements according to the NRC (1994) except Se (**Tables 1 and 2**). SS, L-Se-Met and Nano-Se were premixed in maize and added to the diets at 0.15 mg selenium (Se)/kg to achieve the appropriate treatment levels (**Table 2**). Diets were fed from 1 to 42 d including starter (1–14 d), grower (14–28 d) and finisher (28–42 d). The starting phase (1 to 14 days of age) diets were provided crumbles and the growing and finisher phase (15 to 42 days of age) diets were provided as pellets. The chicks were raised in cages (120 cm×120 cm×50 cm, length×width×height), equipped with nipple waterers and tube feeders. There were 12 chicks per cage, and 4 cages were used for each treatment. All

chicks were given ad libitum access to feed and water. Temperature was maintained at 32 °C for the first week and then gradually reduced according to normal management practices, until a temperature of 23 °C was achieved. During the first week, 24 h of light were provided with a reduction to 20 h afterwards. Dead birds were recorded daily, and chicks weight and feed intake per cage were measured weekly to calculate the average daily gain (ADG), average daily feed intake (ADFI) and feed conversion rate (FCR, feed/gain) during 1 to 42 days of age. At the 42th day of the feeding trial, 2 chicks per treatment were slaughtered by severing the jugular vein. Carcass weight, abdominal cavity fat weight, heart weight and liver weight were measured, immediately processed in liquid nitrogen and then stored at -70 °C.

The effects of dietary Se source and level on immunity system, lymphatic organs and Total antioxidant activity

Effects on antibody against SRBC¹ influenza and Newcastle and lymphatic organs

At Day 14, two birds per pen were randomly selected for a primary antibody response to SRBC. A 1.0-mL suspension (7% vol/vol) of

SRBC was injected intraperitoneally. At Day 21, birds were bled via cardiac puncture to collect serum. Blood serum was frozen until analysis for antibody titers to SRBC could be performed [4].

Although BW of chicks was measured at Day 18, chicks remained in batteries and had ad libitum access to treatment diets and water until serum was collected at Day 21. Differential blood cell counts were obtained from two birds per pen at Day 15. Blood was drawn via cardiac puncture and then smeared on a glass slide by using the beveled edge of an additional glass slide. Glass slides containing blood smears were stained with Wright's Stain Pack². Percentage myeloid and mononuclear cells were counted microscopically by using a 100X oil immersion objective. Immune organ weights were obtained from the same two birds per pen used to quantify blood differentials. Birds were weighed and killed by CO₂ inhalation. The lymphatic organs (bursa and spleen) were dissected out and weighed immediately.

Vaccinations to Newcastle and influenza disease viruses were administered at Day 1 via coarse spray in the commercial hatchery. At Day 15, plasma was collected from one bird per pen after birds were bled via cardiac

¹ - Sheep Red Blood Cell

² - Fisher Diagnostics, Swedesboro, NJ 08085.

puncture. Plasma was frozen until antibody titers for Newcastle and influenza disease viruses could be performed. Serum samples were thawed at room temperature and diluted 500-fold (1:500) in diluent. Diluted serum was added (100 μL) to 96-well plates coated with Newcastle and influenza disease viruses. Plates were covered and allowed to incubate at room temperature for 30 min. After incubation, plates were aspirated and washed with 350 μL of sterile distilled water. A multichannel pipette was used to dispense 100 μL of the conjugate and plates were allowed to incubate at room temperature for 30 min. Substrate was then dispensed (100 μL) in the wells to facilitate a color reaction as plates were allowed to incubate at room temperature for an additional 15 min. A stop solution³ was then added to end the enzymatic process. Plates were read on a Microplate Reader at 650 nm for determination of Newcastle and influenza antibody titers.

Effects on total antioxidant activity

Total antioxidant activity was determined using a commercial kit (Antioxidant assay kit, cat. No. CS0790; Sigma-Aldrich, St. Louis, MO, USA). Absorbance was read at 405 nm using a Multiscan EX microplate reader (Labsystems, Helsinki, Finland). Under

conditions of this assay, the volume of plasma was 10 μL and total antioxidant activity values of samples were expressed as an equivalent of themmol concentration of a (\pm)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) solution. Trolox standard curve at the range of 0.00 – 0.42 mM was prepared for the assay.

Statistical analysis

Treatments were analyzed as a factorial design 2^3 under the general model:

$$Y_{ijk} = \mu + T_{ijk} + e_{ijk}$$

$$T_{ij} = A_i + B_j + C_k + (AB)_{ij} + (AC)_{ik} + (BC)_{jk} + (ABC)_{ijk}$$

Where Y_{ijk} is the dependent variable; μ is the general mean; T_{ijk} is the treatment 1, 2, 3, 4, 5, 6; and e_{ijk} is the experimental error, calculated using the GLM procedure of the SAS software [19]. The broilers were the experimental units for all analyses. Treatment means were compared using the Duncan method, an α -value of 0.05 was used to assess significance and orthogonal polynomial contrast were performed to find a linear or quadratic response.

RESULTS

The effects of dietary Se source and level on growth performance, Feed conversion ratio, carcass component and lymphatic organs

³ - IDEXX Laboratories, Westbrook, ME 04092.

As shown in Table 3, the group unsupplemented with any forms of Se showed the least growth performance and carcass component. In the beginning, initial BW did not differ significantly across the treatment groups. However, significant differences ($P < 0.05$) were observed in final ADW, ADFI and ADG of groups supplemented with Nano-Se compared with the two other groups. When Mineral-Se, Organic-Se and Nano-Se were added to the diet, ADFI, ADG, ADW and FCR showed better performance at the Se concentration of 0.5, 0.2 and 0.5 mg/kg respectively. Nano-Se at the Se concentration of 0.5 mg/kg showed the best results about ADFI, ADG, ADW and FCR. ADFI, ADG, ADW and FCR were affected by Se source, Se level and Se level \times Se source interaction ($P < 0.05$). Significant differences ($P < 0.05$) were observed in final liver and abdominal cavity fat weight of groups supplemented with Nano-Se compared with the control groups, however no significant differences were observed in heart, carcass, bursa of fabricius and spleen weight.

The effects of dietary Se source and level on immunity system and total antioxidant activity

Chickens supplemented with Nano-Se showed significantly higher immunity system

and total antioxidant activities ($P < 0.05$) in the serum than did those in the control groups (Table 4). When Mineral-Se, Organic-Se and Nano-Se were added to the diet, immunity system and total antioxidant activities showed better performance at the Se concentration of 0.5 mg/kg.

DISCUSSION

The natural Se content of grain and forages used in poultry feedstuffs is only 0.02 to 0.12 mg/kg, with values more commonly at the lower end of this range [25]. Intake of such feeds may result in a serious Se deficiency, with subsequently impaired poultry efficiency, health problems, or both. The basal diet used in this experiment contained only 0.03 mg Se/kg diet which was far lower than the requirements. This is characterized by significant decreases in final BW and DWG; thus, the results demonstrated that Se was an essential micronutrient for chickens. However, Haug et al. [9] found no signs of Se deficiency for 21 d old male broilers fed different levels of Se (0.037 to 0.130 mg/kg). This indicated that the number of days chickens were supplemented was one of the most important factors in evaluating whether they were Se deficient. Upton et al. [22] reported significant increases in the BW of 42-d old broilers when they were given diets

supplemented with 0.2 mg/kg of organic Se, as compared with a diet supplemented with inorganic Se and a control diet (no supplemented Se). Similar results were observed in other studies (Ševčíková et al. 2006; Dlouhá et al. 2008), and results of the present study also clearly indicated that Se provided via a supplemented diet could improve the final BW and DWG of chickens. In contrast, several reports have shown that dietary supplementation with inorganic Se, organic Se, or both don't affect the BW and DWG of chickens [6, 16, 5]. This result might be associated with the high dietary background levels of Se and the fact that the levels of Se in the control diet could have masked the effect of supplemental Se in previous experiments. It was found that Nano-Se had similar or higher bioavailability and much less toxicity in mice, rat, broiler and goat compared with selenite [8, 10, 21]. In our study, FCR was improved when birds were given diets with Nano-Se, and this implied that an additional requirement for Se was necessary for chickens. This result was observed in several other studies [14, 17] in which improved FCR was reported. In addition, different results for final BW, DWG, and FCR were observed with different concentrations of dietary Nano-Se. This

suggests that feeding a diet containing 0.40-0.50 mg/kg of Nano-Se produced the greatest improvement in chickens for the cost according to the present results.

In the body, Se joins to proteins and form seleno-proteins. These compounds contain antioxidant properties. On the other hand, glutathione peroxidase that destroys peroxides contains Se and is important in defense against oxidant factors along with Superoxide dismutase and katalaz enzymes [11].

As we know, the liver is one of the most prolific organs in the body and is affected by sickening factors at different ways. As for antioxidant role of Se and importance of glutathione peroxidase enzyme in autoxidation defense of broilers, it can be concluded that use of Se in diet protect liver against sickening factors. Therefore liver weight loss may be because of this reason. As for Se roles in liver, activities of liver and its weight decrease. On the other hand, liver weight loss can be attributed to decrease of abdominal cavity fat, because when the adipose tissue of body decrease, reserve of fat in liver reduces and consequently liver weight reduces.

Studies show that in situation of stress, hypertrophy and increase of liver weight is raised from fat aggregation in it and

supplementation of diet with antioxidant compounds like Se maybe improve liver function [12]. These results are in agreement with our findings.

Because of presence of Se, digestion, absorption and metabolism of fat are done naturally and maybe decrease abdominal cavity fat. In agreement with our study, Robert et al [18] reported that Se addition to the diet did not affect weight of spleen and bursa of fabricius significantly.

It could be concluded from this study that Nano-Se supplementation of chicken diets was effective in increasing the growth performance and FCR of chicken and improving immune system. A recommendation for nano-Se supplementation of broiler diets of 0.3 to 0.5 mg/ kg is supported by these findings.

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Table 1: Ingredients and nutrient composition of the basal diets

Ingredients (g/kg)	Starter 0-14 days	Grower 14-28 days	Finisher 28-42 days
Maize	54.24	58.43	64.2
Soybean meal, crude protein 465 g/kg	39	35.5	30
Soybean oil	1.6	1.6	1.6
Dicalcium phosphate	2.2	0.2	1.8
Limestone	1.2	0.1	0.1
Vitamin–mineral premix ^a	0.5	0.5	0.5
DL-Methionine, ≥985 g/kg	0.4	0.31	0.27
L-Lysine	0.28	0.15	0.13
L-Threonine	0.09	0.04	0.02
Sodium chloride	0.27	0.27	0.28
Choline	0.13	0.2	0.2
Analyzed composition as fed basis			
Metabolizable energy (Kcal/kg)	2870	2925	2990
Dry matter content	92.28	92.21	92.24
Crude protein	22.5	21	19
Lysine	1.43	1.24	1.09
Methionine + Cysteine	1.07	0.95	0.86
Calcium	1.05	0.9	0.85
Total phosphorus	0.5	0.45	0.42

a Provided per kilogram of diet: vitamin A 1500 IU, vitamin D3 200 IU, vitamin E 10 IU, vitamin K3 0.5 mg, vitamin B12 0.01 mg, biotin 0.15 mg, choline; 1100 mg, folic acid 0.55 mg, niacin 30 mg, pantothenic acid 10 mg, pyridoxine 3.5 mg, riboflavin 3.5 mg, thiamine 1.8 mg, copper 8 mg, iodine 0.35 mg, iron 80 mg, manganese 60 mg, zinc 40 mg.

Table 2: The analyzed Se contents (mg/kg) in all diets

Diet	0	SS		L-Met-Se		Nano-Se	
	0.00	0.2	0.5	0.2	0.5	0.2	0.5
Starter	0.034	0.231	0.532	0.230	0.535	0.2230	0.524
Grower	0.032	0.226	0.529	0.228	0.533	0.225	0.528
Finisher	0.031	0.232	0.531	0.232	0.537	0.2234	0.532

Table 3: Effects of dietary Se source and level on growth performance, Feed conversion ratio and carcass component. ^A

Added Se Mg/kg		SS		L-Met-Se		Nano-Se		P-value Se source	P-value Se level	P-value Se level × source
	0	0.2	0.5	0.2	0.5	0.2	0.5			
ADFIB										
0-14 d	23.6c±0.45	26.3bc ± 0.73	27.8ab ± 0.78	27.2ab ± 1.42	25.4bc ± 0.62	25ab ± 0.22	29.2a ± 1.53	0.004	0.001	0.123
14-28 d	63.1 ± 3.52	66.1 ± 2.81	71.1 ± 4.09	67.9 ± 2.53	64.9 ± 1.84	67 ± 2.75	71.2 ± 3.15	0.457	0.378	0.863
28-42 d	175.7c ± 4.34	180.2bc ± 2.12	186.7ab ± 5.47	180.1bc ± 2.76	178bc ± 2.68	179.2bc ± 2.25	191.8a ± 2.27	0.003	0.005	0.423
0 – 42 d	86.6c ± 2.21	91.2abc ± 1.76	92.6ab ± 2.25	91.7abc ± 1.16	89.4bc ± 1.74	90.4abc ± 1.06	97.4a ± 2.06	0.002	0.001	0.256

ADGC										
0 – 14 d	19.8bc ± 0.2	21bc ± 0.5	21.2bc ± 0.92	20.6ab ± 0.16	20.2bc ± 0.4	22.9ab ± 0.87	24.7a ± 0.23	0.002	0.001	0.412
14 – 28 d	39.1b ± 1.36	43.6bc ± 0.83	42.9bc ± 0.88	44.4ab ± 0.42	43bc ± 0.06	44.6ab ± 1.6	46.5a ± 1.15	0.745	0.001	0.786
28 – 42 d	76.6b ± 1.97	75.8b ± 21.61	81.3ab ± 1.9	81.9ab ± 2.16	79.4bc ± 4.6	83.4a ± 2.66	85.5a ± 2.04	0.135	0.004	0.423
0 – 42 d	44.7c ± 0.71	46.3b ± 0.68	48bc ± 0.46	48.9bc ± 0.8	47.5bc ± 1.56	50.3ab ± 1.43	52.2a ± 1.06	0.027	0.001	0.543
ABWD										
1 d	38 ± 0.98	39 ± 0.88	39 ± 0.26	40 ± 0.89	39 ± 0.57	38 ± 0.2	38 ± 0.45	0.06	0.07	0.07
14 d	440c ± 3.98	458abc ± 6.88	466ab ± 10.26	443b ± 2.89	447bc ± 6.57	453abc ± 15.2	469a ± 2.45	0.004	0.002	0.386
28 d	1400c ± 15.5	1480abc ± 14.68	1481abc ± 7.3	1418b ± 8.47	1418b ± 12.75	1486ab ± 26.12	1500a ± 16.7	0.005	0.003	0.265
42 d	2295c ± 56.85	2345bc ± 28.84	2352bc ± 33.81	2369b ± 105.8	2336b c ± 21.22	2436ab ± 2.7	2470a ± 13.32	0.002	0.001	0.465
Feed Conversion Ratio										
0-14 d	1.27 ± 0.04	1.25 ± 0.02	1.23 ± 0.03	1.25 ± 0.04	1.25 ± 0.04	1.25 ± 0.03	1.24 ± 0.05	0.365	0.324	0.687
14-28 d	1.66 ± 0.08	1.64 ± 0.03	1.57 ± 0.03	1.56 ± 0.04	1.63 ± 0.03	1.59 ± 0.03	1.58 ± 0.06	0.487	0.256	0.476
28-42 d	2.31 ± 0.08	2.29 ± 0.05	2.25 ± 0.07	2.2 ± 0.05	2.23 ± 0.01	2.25 ± 0.07	2.21 ± 0.06	0.587	0.276	0.587
0 – 42 d	1.73 ± 0.04	1.7 ± 0.01	1.69 ± 0.009	1.67 ± 0.03	1.7 ± 0.05	1.69 ± 0.02	1.67 ± 0.01	0.358	0.476	0.879
Carcass component in 42 dE										
Carcass	70.07 ± 0.47	70.04 ± 0.28	70.8 ± 0.25	70.22 ± 0.64	70.42 ± 0.66	70.13 ± 0.47	70.8 ± 0.42	0.145	0.278	0.476
Liver	2.55a ± 0.07	2.35b ± 0.04	2.31b ± 0.06	2.37b ± 0.03	2.29b ± 0.07	2.34b ± 0.06	2.25b ± 0.04	0.045	0.001	0.887
Heart	0.47 ± 0.01	0.50 ± 0.02	0.47 ± 0.01	0.49 ± 0.02	0.49 ± 0.01	0.48 ± 0.02	0.49 ± 0.02	0.343	0.487	0.789
Abdominal cavity fat	1.35a ± 0.09	1.19ab ± 0.14	1.07ab ± 0.10	0.93b ± 0.07	1.24ab ± 0.11	1.21ab ± 0.12	1.02b ± 0.13	0.047	0.004	0.365
lymphatic organs in 42 dE										
Bursa of fabricius	0.19 ± 0.013	0.19 ± 0.011	0.19 ± 0.021	0.19 ± 0.016	0.20 ± 0.014	0.20 ± 0.009	0.21 ± 0.024	0.389	0.378	0.764
spleen	0.09b/ ± 0.007	0.11ab ± 0.006	0.12a ± 0.005	0.11ab ± 0.011	0.10ab ± 0.011	0.12a ± 0.010	0.12a ± 0.010	0.287	0.007	0.589

					0.003				
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a-c Means within a row with different letters differ significantly ($P < 0.05$); Results are presented as means \pm SD of triplicate observations; A Data represent mean values of six replicate of each treatment; B Average daily feed intake; C Average daily Gain; D Average body weight; E percent of live weight

Table 4: Effects of dietary Se source and level on immunity system and Total antioxidant activity in 42 d

Added Se Mg/kg	Mineral- Se		Organi c-Se		Nano-Se		P- value Se source	P- value Se level	P- value Se level \times source	
	0	0.2	0.5	0.2	0.5	0.2				0.5
Immunity system										
Antibody against influenza	4.75 ^c \pm 0.16	5 ^{bc} \pm 0.26	5.50 ^{ab} \pm 0.18	5.50 ^{ab} \pm 0.18	5.25 ^{abc} \pm 0.16	5.25 ^{abc} \pm 0.31	5.62 ^a \pm 0.18	0.001	0.004	0.897
Antibody against Newcastle	5 \pm 0.18	5.12 \pm 0.22	5.35 \pm 0.26	5.12 \pm 0.22	5.37 \pm 0.26	5.25 \pm 0.25	5.50 \pm 0.18	0.674	0.348	0.675
Antibody against SRBC ^a	8 ^c \pm 0.42	8.62 ^{abc} \pm 0.26	9.12 ^{ab} \pm 0.29	8.37 ^{bc} \pm 0.18	9.25 ^a \pm 0.25	8.75 ^{ab} \pm 0.31	9.87 ^a \pm 0.22	0.006	0.198	0.267
Total antioxidant activity	0.71 ^b \pm 0.03	0.74 ^b \pm 0.08	0.81 ^{ab} \pm 0.06	0.80 ^{ab} \pm 0.06	0.78 ^{ab} \pm 0.07	0.83 ^{ab} \pm 0.02	0.94 ^a \pm 0.04	0.009	0.006	0.586

^{a-c} Means within a row with different letters differ significantly ($P < 0.05$); Results are presented as means \pm SD of triplicate observations.