

Role of Selenium from Different Sources in Prevention of Pulmonary Arterial Hypertension Syndrome in Broiler Chickens

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Abstract Pulmonary arterial hypertension (PAH) syndrome in broilers is associated with hypoxia, which prevails at high altitude. Oxidative stress is the pathogenic mechanism underlying PAH. Because selenium is key element in the structure of antioxidant enzymes, we evaluated pulmonary hypertensive responses in broiler chickens fed with diets supplemented with organic or nano-selenium. One hundred forty-four broilers (starting at 5 days old) were fed with (i) control group: birds received a standard diet; (ii) nano-selenium group: birds were fed with basal diet supplemented with nano-selenium at 0.3 mg/kg; and (iii) organic selenium group: birds received basal diet supplemented with organic selenium at 0.3 mg/kg. We assessed growth performance, carcass characteristics, antioxidant variables, blood parameters, and small intestine morphology. Although Se supplementation did not affect growth performance, carcass traits, and organ weight ($P > 0.05$), the right to total ventricular weight ratio (RV:TV), malondialdehyde concentration in the liver, and heterophil to lymphocyte ratio were significantly lower in the nano-selenium group relative to the control ($P < 0.05$). Chickens that received nano-selenium also elicited significantly higher antibody titers after 24 h of an injection of sheep red blood

cells ($P < 0.05$). Nano-selenium supplementation also significantly increased villus height, absorptive surface area, and lamina propria thickness relative to the control ($P < 0.05$) in different segments of the small intestine. In contrast, organic selenium supplement improved intestinal morphometry only in the jejunum. We conclude that dietary supplementation of 0.30 mg/kg nano-selenium could prevent right ventricular hypertrophy as reflected by reduced RV:TV, reduced levels of lipid peroxidation in the liver, and improved gut function.

Keywords Ascites · Broiler chicken · Nano-selenium · Organic selenium

Introduction

Intensive genetic selection has favored rapid growth of broiler chickens. However, rapid growth, particularly of the breast muscles, has not been paralleled by allometric growth of the heart and lungs [1]. Consequently, an imbalance between oxygen-demanding organs (i.e., muscles) and oxygen-supplying organs (i.e., heart and lungs) has emerged as a contributor to pulmonary arterial hypertension syndrome (PAH, ascites). PAH syndrome in broilers is associated with hypoxia, which exists at high altitude. The pathogenesis of PAH underlies oxidative stress. The susceptibility of broilers to PAH exacerbates under conditions of limited oxygen availability (such as hypobaric hypoxia) or with increased oxygen demands at the tissues (e.g., thermoregulation in cold temperatures). Both these conditions prevail at high altitudes, where the environment is cold and hypoxic. Hence, PAH is the most common metabolic disorder and the major cause of mortality in broiler chickens reared at high altitudes [2, 3]. Physiological responses to sustained hypoxia imposed by high altitude include excessive production of reactive oxygen species (ROS)

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and vascular remodeling in the lung characterized by hypertrophy and hyperplasia of the smooth muscle layer of arterioles [4].

Selenium (Se) is an essential trace element that regulates antioxidant defense mechanisms in all living cells [5]. The role of Se is controlling glutathione (GSH) and its major Se-containing antioxidant enzymes, glutathione peroxidase (GPX) and thioredoxin reductase. Glutathione and GPX protect the integrity of unsaturated bonds of membrane phospholipids by scavenging ROS, which initiate and propagate lipid oxidation [6].

The Se sources vary in their ability to meet the dietary requirement of the animal as its metabolism differs due to chemical forms. Schwarz and Foltz (1957) categorized these sources into three groups [7]: (1) elemental Se, which is poorly absorbed by the animal; (2) inorganic salts, such as selenites and selenates; and (3) organic Se compounds such as selenomethionine. Nowadays, with advances in nanotechnology, a new source—nano-selenium—has attracted widespread attention because nanoparticles exhibit novel characteristics such as high surface activity, catalytic efficiency, and adsorbing ability, as well as low toxicity [8].

Sodium selenite (inorganic form of Se) is the form that is most commonly used in broiler diets. Sodium selenite is toxic and needs to be soluble as ionic form in order to become available in the gastrointestinal tract. However, the electric charges of this ionic form may interact with other diet components (minerals, proteins, and carbohydrates), rendering them partially unavailable to animals [9]. This has recently motivated researchers to study alternative sources of selenium. The effects of nano-selenium in broiler diets on growth performance have recently been investigated, but it is unclear whether nano-selenium, along with other sources, affects PAH in broiler chickens. The aim of this study was to evaluate PAH in broiler chickens fed with diets supplemented with nano- or organic selenium.

Materials and Methods

Birds and Housing

The experiment was carried out in Shahrekord, Iran, at an altitude of 2100 m above sea level. The experimental animals were kept, maintained, and treated according to the accepted standards for the human treatment of animals.

A number of 200-day-old broiler chicks (Ross 308 strain) were raised on a commercial diet until 5 days of age. The runts and out-of-range chicks were removed, and a total of 144 5-day-old broilers were randomized across 12 floor pens (2 m²) with 12 chicks each so that all pens had equal initial body weights (864 ± 10 g). Each pen was equipped with a bell drinker and a feed trough for free access. The house

temperature was set at 32 ± 1 °C on day 1 and declined to 25 ± 1 °C on day 7, 20 ± 1 °C on day 14, 15 ± 1 °C on day 14, and onward until 35 days of age as previously described [10]. Birds were subjected to 23 h light and 1 h dark throughout the trial.

Treatments

Two experimental diets were formulated for the starting (1–21 days of age) and growing/finishing (22–35 days of age) stages according to the NRC (1994) recommendations (Table 1). (i) Control group received the control diet. Two additional diets were made by supplementing the control diet with (ii) nano-selenium and (iii) organic selenium at 0.3 mg/kg. Organic selenium (Sel-Plex) was obtained from Alltech Inc. (St. Louis, USA) and selenium nanoparticles were obtained from American Elements Co. (Los Angeles, USA). The particle size of nano-selenium ranged from 10 to 45 nm with 99.95% purity. The particles had true density of 3.89 g/cm³ with spherical morphology and gray color.

Table 1 Composition of the experimental diets fed to broilers in the starting and growing and finishing stages

Ingredient (% , unless noted)	1–21 days	22–35 days
Yellow maize	57.04	50.25
Soybean meal	37.45	39.16
Oyster shell	1.02	0.98
DL-methionine	0.27	0.18
L-lysine HCl	0.12	–
Vitamin D3	0.1	0.1
Vitamin E	0.1	0.1
Mineral premix ^a	0.25	0.25
Vitamin premix ^b	0.25	0.25
Sodium bicarbonate	0.04	0.04
Sodium chloride	0.31	0.32
Oil	1.25	6.77
Dicalcium phosphate	1.78	1.61
Threonine	0.02	–
Energy (kcal/kg)	2900	3200
Protein	22	22

^a Provided the following per kilogram of diet: Se (from sodium selenite), 0.2 mg; Mn (from MnSO₄·H₂O), 99.2 mg; Zn (from ZnO), 84.7 mg; Fe (from FeSO₄·7H₂O), 50 mg; Cu (from CuSO₄·5H₂O), 10 mg; and I (from Ca(IO₃)₂·H₂O), 0.99 mg

^b Provided the following per kilogram of diet: vitamin A (trans-retinylacetate), 9000 IU; vitamin D3 (cholecalciferol), 2000 IU; vitamin E (*dl*-tocopheryl acetate), 18 mg; vitamin K3, 2 mg; vitamin B1, 1.7 mg; vitamin B2, 6.6 mg; vitamin B3, 0.98 mg; vitamin B5, 29.7 mg; vitamin B6, 2.9 mg; vitamin B12, 0.15 mg; folic acid, 1 mg; and choline chloride, 500 mg

Measurements

Body weight and feed consumption were measured throughout the entire trial (5–35 days) based on each pen. Feed to gain ratios were also calculated and corrected for mortality body weights in the entire trial. At 35 days of age, 12 birds per treatment were selected for blood collection. Blood samples (~3 mL) were collected from the brachial vein and centrifuged at $2500\times g$ for 10 min to obtain sera, which were used to determine nitric oxide (NO) and uric acid (UA).

Nitric oxide (nitrate + nitrite) was measured using methods described by Behrooj et al. [11]. In brief, serum samples were first deproteinized in 75 mmol ZnSO₄ and 55 mmol NaOH. Following centrifugation, the supernatant was diluted with glycine buffer (45 g/L; pH 9.7) and freshly activated cadmium granules (~2 g) were added to the samples. Cadmium granules were rinsed three times in deionized water and 5 mmol CuSO₄ dissolved in glycine was added (NaOH buffer 5 g/L; pH 9.7). Upon continuous stirring for 10 min, the samples were transferred to new tubes and subjected to Griess reaction. Griess reagent 1 (1% sulfanilamide in 5% phosphoric acid) was added to the sample tubes and incubated for 10 min at room temperature while protected from light. Griess reagent 2 (*N*-naphthyl ethylenediamine dihydrochloride in water) was then dispensed into all samples, and the optical density was measured at 540 nm within 10 min by a spectrophotometer (Corning 480, Swedesboro, NJ, USA).

Serum UA concentration was analyzed colorimetrically according to Fossati et al. (1980) [12] using 3,5-dichloro-2-hydroxybenzene sulfonic acid/4-aminophenazone chromogenic system in the presence of horseradish peroxidase and uricase. The red color formation was then measured at 520 nm.

Malondialdehyde (MDA) concentration was measured as an index of lipid peroxidation by thiobarbituric acid-reactive substances, according to methods described by Hassanpour et al. (2014) [13]. Segments of liver tissue were homogenized and sonicated for cell lysate. Trichloroacetic acid was added to the lysate to precipitate proteins and the supernatant was recovered after centrifuging. MDA is formed as a result of lipid peroxidation and reacts with thiobarbituric acid under acidic conditions and high temperature (90–100 °C). The reaction yields a pink MDA-TBA adduct which was measured using a spectrophotometer at 535 nm (Corning 480, USA).

Samples of blood were collected in microhematocrit tubes to measure hematocrit. An aliquot of blood was also obtained on glass slides to prepare a smear for the determination of leukocyte counts. The May-Grunwald and Giemsa stains were used for staining the smears 3 h after methyl alcohol fixation [14]. One hundred leukocytes, including granular (heterophils) and non-granular (lymphocytes), were enumerated and the heterophil to lymphocyte ratio (H:L) was calculated. All chemical reagents were obtained from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA).

After blood collection, birds were euthanized for carcass processing. Data obtained at processing included weights of the hot eviscerated carcass, breast, thigh, liver, spleen, and bursa of Fabricius. The hearts were also harvested and the ventricles were dissected and weighed to calculate the right to total ventricular weight ratio (RV:TV ratio). Moreover, 2-cm segments of the duodenum, jejunum, and ileum were sampled to measure the intestinal morphometric variables (i.e., villus sizes: length, width, surface area, and lamina propria thickness). After fixing the intestinal segments in Clark solution, each segment was divided into three sections along its length. Sections were stained by periodic acid Schiff (PAS) reagent, and then, rows of villi were separated, transferred to glass slides, and covered with a cover slip. Measurements were made at $\times 1000$ magnification. The villus length and width were used to calculate villus surface area according to the formula $\pi \times (\text{villus width}) \times (\text{villus length})$, as described by Sakamoto et al. [15]. The lamina propria thickness was measured at the space between base of the villus and top of the muscularis mucosa.

We measured antibody titers against sheep red blood cell (SRBC) in a hemagglutination assay as humoral immunity. SRBC titers were determined according to a protocol adapted from Bartlett and Smith (2003) [16].

Statistical Analysis

Data were analyzed in a completely randomized design according to the GLM procedure of SAS (2007). Means were separated by Duncan's multiple range test.

Results

Both nano- and organic selenium did not affect feed intake, weight gain, and feed to gain ratio in different stages (Table 2). Nevertheless, birds that received nano-selenium had the lowest feed to gain ratio.

Carcass, breast, and thigh yields were similar between nano-selenium, organic selenium, and control groups ($P > 0.05$) (Table 3). The weights of the liver, spleen, and bursa of Fabricius relative to body weight were also similar among the treatment groups ($P > 0.05$). However, the right ventricular weight ratio (RV:TV) was significantly lower in the nano-selenium group relative to the control ($P < 0.05$) and a similar trend was observed in birds fed with organic selenium but this was not significantly different.

Both sources of selenium increased serum NO compared to the control though the difference was insignificant, but only nano-selenium significantly reduced MDA concentration in the liver and H:L ratio compared to the control ($P < 0.05$) (Table 4). Nano-selenium also elicited the greatest humoral immune response to SRBC injection after 24 h, though HI

Table 2 Effects of nano- and organic selenium on growth performance of broiler chickens

	Control	Nano-Se	Organic Se
3–21 days			
Weight gain (g/bird)	672 ± 53.34	638 ± 48.79	686 ± 53.58
Feed intake (g/bird)	1005 ± 48.16	938 ± 86.34	990 ± 58.79
Feed:gain	1.5 ± 0.049	1.47 ± 0.038	1.45 ± 0.067
22–35 days			
Weight gain (g/bird)	906 ± 39.94	1031 ± 28.80	904 ± 74.63
Feed intake (g/bird)	1899 ± 93.54	2029 ± 40.13	1847 ± 108.43
Feed:gain	2.09 ± 0.053	1.97 ± 0.084	2.05 ± 0.067
3–35 days			
Weight gain (g/bird)	1579 ± 47.47	1669 ± 50.102	1590 ± 109.22
Feed intake (g/bird)	2903 ± 117.29	2968 ± 107.19	2837 ± 164.96
Feed:gain	1.83 ± 0.029	1.78 ± 0.060	1.79 ± 0.059

titers following 7 days of SRBC injection remained unchanged across dietary treatments ($P < 0.05$) (Table 4). Uric acid and hematocrit were not significantly influenced by dietary treatments.

In all intestinal morphometric parameters (except ileum villus height), nano-selenium supplement significantly increased villus height, villus surface, and lamina propria thickness when compared to the control group ($P < 0.05$) (Table 5). Organic selenium also significantly increased villus height, villus surface, and lamina propria thickness, but only in the jejunum.

Discussion

Supplementation of either nano- or organic selenium had no significant impact on growth performance of broiler chickens similar to findings reported by Choct et al. [17], Payne and Southern [9], and Ryu et al. [18]. There was no significant change in carcass characteristics among dietary treatments. Payne and Southern [9] reported similar findings with Se-

Table 3 Effects of nano- and organic selenium supplementation on carcass characteristics in broiler chickens measured at 35 days of age

	Control	Nano-Se	Organic Se
Carcass yield (%)	65.87 ± 0.80	66.27 ± 0.59	65.93 ± 0.57
Breast yield (%)	35.59 ± 0.44	35.89 ± 0.33	35.73 ± 0.31
Thigh yield (%)	30.48 ± 0.38	29.95 ± 0.28	29.99 ± 0.26
Liver (%)	2.67 ± 0.071	2.55 ± 0.072	2.65 ± 0.084
Spleen (%)	0.089 ± 0.009	0.110 ± 0.008	0.109 ± 0.009
Bursa of Fabricius (%)	0.133 ± 0.007	0.143 ± 0.019	0.154 ± 0.027
RV:TV ratio (g/g)	0.29 ^a ± 0.016	0.24 ^b ± 0.012	0.27 ^{ab} ± 0.017

Means within the same row with different superscripts are significant ($P < 0.05$)

enriched yeast. Moreover, Cai et al. [19] found that broiler immune organ (spleen, bursa, and thymus) index was not affected by nano-selenium supplementation.

Ascetic birds are identified by an RV:TV ratio greater than 0.27 (Ahmadipour et al. [20]). In the present study, nano-selenium, but not organic selenium, reduced the RV:TV ratio to levels below this threshold. Numerous inflammatory cells are present in the heart and other tissues of ascetic birds, and white blood cell activity can also produce reactive oxidants [21]. In this situation, antioxidants have been proven to reduce lung and heart congestion and the resulting pulmonary hypertension syndrome in broilers [22]. Tanguy et al. showed that dietary selenium intake alleviated hypertension in the spontaneously hypertensive rats by increasing the cardiac antioxidant capacity and reducing myocardial cell apoptosis [23]. Nano-selenium is an important antioxidant that is located at the catalytic site of thioredoxin reductase and glutathione peroxidase enzymes [24], reflected in significantly lower MDA concentrations in the liver of birds receiving nano-selenium. It has been reported that nano-selenium could boost hepatic antioxidant capacity with concomitant decline in MDA in broiler chickens exposed to heat stress [25]. MDA is produced as one of the final products of polyunsaturated fatty acid peroxidation in tissue cells and indicates oxidative stress [26]. In this regard, nano-selenium significantly reduced the H:L ratio (an index of stress [27, 28]), suggesting that these birds endured less stress compared to those in the control group.

Higher reaction to SRBC in the nano-selenium group after 24 h is consistent with the results reported by Cai et al. [19], who observed elevated levels of IgG and IgM with Se supplementation. Similarly, humoral and cellular immunity were positively affected by supplementing 0.3 mg/kg nano-selenium in layer chicks [29]. Safdari-Rostamabad et al. observed significantly higher antibody responses to SRBC by supplementing 1.2 mg/kg nano-Se of heat-stressed broilers [30]. Selenium plays an important role in immunomodulation

Table 4 Effects of nano- and organic selenium on antioxidant variables and blood parameters in broiler chickens measured at 35 days of age

	Control	Nano-Se	Organic Se
Serum nitric oxide ($\mu\text{mol/L}$)	36.72 \pm 2.59	43.47 \pm 4.59	42.10 \pm 11.31
Serum uric acid (mg/dL)	4.73 \pm 0.60	4.54 \pm 0.70	3.46 \pm 0.99
Liver malondialdehyde ($\mu\text{mol/L}$)	0.99 ^a \pm 0.17	0.35 ^b \pm 0.04	0.70 ^{ab} \pm 0.38
Heterophil to lymphocyte (%)	0.92 ^a \pm 0.07	0.70 ^b \pm 0.04	0.75 ^{ab} \pm 0.04
Hematocrit (%)	43.3 \pm 1.33	42.9 \pm 1.13	41.4 \pm 1.60
HI titers after 24 h of SRBC inj.	1.71 ^a \pm 0.47	3.28 ^b \pm 0.42	2.5 ^{ab} \pm 0.67
HI titers after 7 days of SRBC inj.	4.67 \pm 0.61	5.4 \pm 0.81	4.8 \pm 0.58

Means within the same row with different superscripts are significant ($P < 0.05$)

against stressors, and nano-selenium acts as a multifunctional antioxidant in supporting oxidative homeostasis in cells, which is called “biological response modifier” [31].

Intestinal morphologic parameters were significantly influenced by nano-selenium supplementation in different segments of the small intestine. Safdari-Rostamabad et al. observed significantly greater jejunal villus height and crypt depth with 1.2 mg/kg nano-selenium supplementation in broiler chickens [30]. Dietary selenium affects both composition of the intestinal microflora and colonization of the gastrointestinal tract, which in turn influence the host selenium status and selenoproteome expression [32]. Harmful oxygen and nitrogen free radicals are produced during Se deficiency, while the antioxidant capacity in the intestine reduces, which together result in oxidative damage to chicken intestinal tissues [33]. Santos et al. (2005) demonstrated the correlation between gut development and reduction of ascites syndrome in broilers [34]. The gastrointestinal tract (GIT) is a highly active organ that has considerable nutrient and oxygen requirements [35]. The GIT and cardiopulmonary system are dependent upon each other, but the relationship can be negatively influenced by inflammation, pathogens, environment, or a high metabolism, resulting in ascites [36]. The high

oxygen demand of the gut may explain why feed restriction can reduce the incidence of ascites in broilers [36].

Ahmadipour et al. (2015b) reported that dietary antioxidants protect enterocytes from apoptotic oxidative stress and improve their growth and development [37]. The small intestine is the main place for digestion and absorption of nutrients. The structure and the function of the intestinal mucosa, which are requisite for intestinal homeostasis, depend upon the balance between apoptosis and proliferation of enterocytes [38]. The increased villus height in our study could be due to the antioxidant effect of selenium, which may delay apoptosis and increase enterocyte viability. In view of the condition of the present study (high altitude of 2100 m), broiler chickens were exposed to hypobaric hypoxia. Considering the fact that the gastrointestinal tract has a high oxygen demand, hypoxia may inhibit gut development and function if exogenous antioxidants are not added to broiler diets.

We conclude that supplementation of 0.30 mg/kg nano-selenium to the diet could help birds prevent PAH syndrome by reducing the RV:TV ratio. Mechanisms underlying this include decreased lipid peroxidation in the liver, immunomodulation, and improvement in intestinal villus morphology.

Table 5 Effects of nano- and organic selenium on morphologic measurement of the small intestine (duodenum, jejunum, and ileum)

	Control	Nano-Se	Organic Se
Duodenum			
Villus height (mm)	1.368 ^a \pm 0.026	1.619 ^b \pm 0.052	1.478 ^{ab} \pm 0.070
Villus surface (mm^2)	1.674 ^a \pm 0.17	2.463 ^b \pm 0.22	2.446 ^b \pm 0.20
Lamina propria depth (mm)	0.221 ^b \pm 0.005	0.300 ^a \pm 0.011	0.248 ^b \pm 0.009
Jejunum			
Villus height (mm)	1.023 ^a \pm 0.043	1.273 ^b \pm 0.051	1.232 ^b \pm 0.068
Villus surface (mm^2)	1.049 ^a \pm 0.15	1.748 ^b \pm 0.19	1.591 ^b \pm 0.16
Lamina propria depth (mm)	0.185 ^a \pm 0.009	0.323 ^c \pm 0.012	0.232 ^b \pm 0.013
Ileum			
Villus height (mm)	0.572 \pm 0.032	0.606 \pm 0.029	0.660 \pm 0.024
Villus surface (mm^2)	0.411 ^a \pm 0.056	0.658 ^b \pm 0.046	0.445 ^a \pm 0.036
Lamina propria depth (mm)	0.180 ^a \pm 0.009	0.219 ^b \pm 0.011	0.203 ^{ab} \pm 0.007

Means within the same row with different superscripts are significant ($P < 0.05$)

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