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**RESEARCH ARTICLE**

# Effect of selenium sources on growth performance, carcass criteria and physical meat quality of broiler chickens

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## Abstract

The present study was conducted to examine the effect of selenium sources on growth performance, carcass criteria and physical meat quality of broiler chickens. A total of 192 One-day old broiler chickens were randomly distributed into four equal treatment groups. Treatment groups were fed a control diet, a control diet supplemented with selenomethonine (0.3 mg /kg), control diet supplemented with Sodium-Selenite (0.3 mg /kg), or control diet supplemented with Nano-Selenium (0.3 mg /kg). The feeding trial lasted for 35 days. Each treatment had six replicates with eight birds each. Broilers fed the diets supplemented with selenium sources increased body weight and body weight gain and improved ($P<0.05$) feed conversion ratio than those fed the control diets. Broilers fed the diets supplemented with Nano-Selenium had higher body weight and body weight gain and lower ($P<0.05$) feed conversion ratio than those fed the control diets or diets supplemented with selenomethonine and sodium selenite. Additionally, broilers fed the diets supplemented with different sources of selenium at 0.30 mg/kg had improved meat quality in leg muscle than those fed the control diets. Furthermore, broilers fed the diet supplemented with different sources of selenium improved dressing percentage and abdominal compared to control, but no differences ($P<0.05$) were observed in internal organs among treatments. Overall, Nano-selenium resulted in best performance. The results from the present study indicated that supplemental selenium improved the growth performance, physiochemical meat quality and carcass criteria of broilers; and the Nano-selenium was more effective than the Se from selenomethonine and sodium-selenite.

**Keywords:** Broilers; Nanotechnology; Meat quality; Performance; Selenium.

## 1. Introduction

The Food and Agriculture Organization of the United Nations (FAO) expects by 2050 that the annual demand for meat products to increase by 76% compared with 2005 levels (Alexandratos and Bruinsma, 2012). Increasing food production to feed the world will be a major challenge. Despite all of these areas of potential growth, experts warn that the supply of food might be insufficient to meet future demand, generating price spikes and social and political instability (MoD, 2014).

Recently, and to achieve cost-effective production, feed additives in different forms (various sources, forms, formulations, etc.) is aimed for improving poultry growth and conversion ratios, and obtaining better quality and value-added products (Gangadoo *et al.*, 2016). Also, dietary supplementation with these mineral additives has a vital role in rapid growth and refining the feed conversion ratio (FCR), so

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lowering the amount of needed feed to attain market weight (Zhao *et al.,* 2017). These elements are vital to the health of poultry since they play important roles in the function of co-enzymes (Peters *et al.,* 2016).

In this respect Selenium (Se) plays a vital role in animal development and various physiological processes (Avery and Hoffmann, 2018). It has been defined as an essential element for growth (Yoon *et al.,* 2007; Wang and Xu, 2008), antioxidant (Peng *et al.,* 2007; Zhou and Wang, 2011), immune competence (Cai *et al.,* 2012; Liao *et al.,* 2012), and reproductive functions, immunocompetence, and ageing (Sevescova *et al.,* 2006; Leeson *et al.,* 2008) of broilers. Selenium Se is involved in the synthesis of at least 30 selenoproteins, that are important in regulating various functions of the body such as antioxidant defense and maintaining intracellular redox balance (Surai and Fisinin, 2014; Surai *et al.,* 2018).

During broiler growth a daily dose of about 0.15 mg/kg is required (National Research Council, 1994). And, the maximum amount of selenium supplemented to animal diets is limited to 0.3 mg/kg in the United States (Anon, 1987), while in other parts such as the European Union; the maximum amount approaches 0.5 mg/kg of diet (Anon, 2012).

In biological systems, free radicals under stress conditions can damage the phospholipid membranes of the cells and destroy the oxidants and antioxidants balance (Wiseman and Halliwell, 1996). The antioxidant effect of Se has been shown by its physiological activities in the forms of selenoproteins, including superoxide dismutase (SOD), GPx, glutathione reductase, selenoprotein P, and selenoprotein in mammals (Kaushal and Bansal, 2007). Many researches proved the role of Se can in regulating the growth performance, reproduction performance, and antioxidant and immune functions of organisms (Mahan and Peters, 2004; Mikulski *et al.,* 2009). It also indicated that Se deficiency in chickens diet, leads to exudative qualities, pancreatic dystrophy, muscular dystrophy, and immunosuppression (Habibian *et al.,* 2015). The efficiency of Se source in meeting the demand for nutrition in poultry depends mainly on its form. Generally, there are two known forms available i.e. organic and inorganic. Inorganic forms of Se are available as selenite, selenate, and selenide, while organic forms are selenomethionine, Se enriched yeast, and Se enriched alga (Sevescova *et al.,* 2006). Using inorganic selenium may exhibits significant limitations that include potential toxicity, poor absorption, interaction with other minerals and dietary components, storage loss, low efficiency of transfer to meat and eggs, inability to supply and maintain selenium reserve in the body. So, the use of these inorganic sources (sodium selenite) is recently debated (Surai, 2000; Pehrson, 1993). Because of these debates and the other limitations, organic selenium in the form of SeMet and selenium enriched yeast is used in nutritional supplements due to their wide bioavailability and lower toxicity among various selenium forms (Schrauzer, 2003).

Compared to inorganic Se, organic Se forms has shown a more enhanced concentration in the tissue, while has no other effects on plasma GPx activity, carcass characteristics and growth performance (Sevescova *et al.,* 2006; Yoon *et al.,* 2007). Food and Drug Administration (FDA, 2000) demonstrates the use of Se yeast as an organic Se in poultry diets, and then Se yeast extracted from various yeast species through different methods (Yoon *et al.,* 2007). When yeast and alga cultivated in a media enriched by Se, they may convert Se to selenomethionine as a source of organic Se which is more efficiently absorbed and retained in tissues compared to inorganic Se salts such as sodium selenite (Yoon *et al.,* 2007). Therefore, identifying an organic Se source with high bioavailability and low toxicity to replace inorganic Se is the first task in poultry nutrition in the future (Surai and Kochish, 2019). On the other hand, to improve both of quality and quantity of livestock production, nanotechnology plays an important role as has been implicated in different aspects of their products (Huang *et al.,* 2015). Using nanotechnology recorded a noticeable improvement in performance parameters of poultry (Panea *et al.*, 2014). Nanoparticles (NPs) typically between 1-100 nm (or more appropriately, 0.2-100.0 nm) have novel properties compared to the bulk material as large surface area, higher surface reactivity, stability, bioactivity, bioavailability, controlled particle size, controlled release of drugs, and site-specific targeting (Youssef *et al.,* 2019). These NPs have antimicrobial properties and the ability to reduce the antibiotic residues in poultry products; so, they could be used to combat and treat antibiotic-resistant bacteria, especially in humans (Verma, Singh, and Vikas, 2012; Hassanen and Ragab, 2020). Furthermore, biodegradable polymers of NPs induced potent immune responses after application as adjuvants or carriers in the mucosal types of poultry vaccines (Jin *et al.,* 2019). Currently, NPs also have been used as accurate, fast and cost-effective diagnostic tools for early detection of avian pathogens (Chen and Neethirajan, 2015). However, concerning the potential toxicity and side effects of using NPs, there is a lack of adequate information regarding the hazardous effects of NPs applications. In addition, the absence of full evaluation criteria of the using outputs of these particles (Patra and Lalhriatpuii, 2020).

Currently, nano-elemental Se has attracted attention by its high bioavailability and low toxicity because of its novel characteristics, such as great surface area, high surface activity, a lot of surface active centers, high catalytic efficiency and strong adsorbing ability and low toxicity of routine Se0 (Wang *et al.,* 2007; Zhang *et al.,* 2008). Since surface area-to-volume ratio increases with decreasing particle size, selenium nanoparticles have high biological activity (Zhang *et al.,* 2005), including anti-hydroxyl radical property (Gao *et al.,* 2002) and a protective action against the oxidation of DNA (Huang *et al.,* 2003). Furthermore, Zhang *et al.* (2005) reported that nano Se possessed higher efficiency than selenite, selenomethionine, and methylselenocysteine (Zhang *et al.,* 2008; Wang *et al.,* 2007) in upregulating selenoenzymes in mice and rats and exhibited lesser toxicity (Zhang *et al.,* 2001). Therefore, the present study was conducted to examine the effect of selenium sources on growth performance, carcass criteria and physical meat quality of broiler chickens

## 2. Materials and Methods

### *2.1. Experimental design and dietary treatments*

During the experiment, the birds were housed and handled according to the South Valley University Institutional Animal Care Committee's recommendations. Chicks were kept in a closed housing in a three-tier wire floor battery cages. Chicks in each replicate were placed in cages with an iron slatted bottom. The cages had sizes of 120, 70, and 50 cm in length, width, and height, respectively. During the trial, the chicks had unrestricted access to feed and water. A total of 192 one –day-old, unsexed broiler chicken (cub 500) were assigned to four treatments diets with each treatment being applied to 6 replicates of 8 chicks. A total of 192 One-day old broiler chickens were randomly distributed into four equal treatment groups. Treatment groups were fed a control diet, a control diet supplemented with selenomethonine (0.3 mg /kg), control diet supplemented with Sodium-Selenite (0.3 mg /kg), or control diet supplemented with Nano-Selenium (0.3 mg /kg). The feeding trial lasted for 35 days. Each treatment had six replicates with eight birds each. The diets were formulated to meet Ross 308 broiler recommendations. Chicks were full access to feed and water during the experimental period. All chicks were kept under the same management guidelines and the environment was kept at a temperature of 34°C for the first week, gradually dropping to 24°C by the fourth week and afterwards. Birds were fed commercial diets according to Cub broilers recommendations to meet the nutrient requirements (Table 1) for starter (1-21 d) and grower (22-35 d) phases, respectively.

### *2.2. Broiler performance parameters*

From the beginning to the end of the experiment, the body weight of the birds in each pen was noted on a weekly basis. The day the birds were weighed also included a measurement of feed residue to determine the amount of feed consumed in each pen. The amount of feed consumed by the weight gained in each pen was divided to obtain at the feed conversion ratio. The magnitude of production variables such as feed consumption and body weight were adjusted appropriately for the dying birds.

**Table 1.** Chemical composition of basal diet (as-fed basis)

| Ingredients (%) | Starter diet | Grower diet |
|---|---|---|
| Corn, ground | 27.59 | 30.00 |
| Sorghum, ground | 27.59 | 30.00 |
| Soybean meal (44% CP) | 28.50 | 25.00 |
| Corn gluten meal (60% CP) | 9.50 | 6.00 |
| Vit & Min. Premix[a] | 0.30 | 0.30 |
| Sunflower oil | 3.00 | 5.52 |
| Dicalcium phosphate | 2.00 | 1.80 |
| Limestone | 1.00 | 1.00 |
| Salt | 0.38 | 0.38 |
| DL-methionine | 0.04 | --- |
| L- lysine HCl | 0.10 | --- |
| Total | 100 | 100 |
| Nutrient Analysis | | |
| ME (kcal/ kg diet) | 3000 | 3187 |
| Crude protein (g/kg) | 236.7 | 204.6 |
| Calcium (g/kg) | 10.0 | 10.0 |
| Available phosphorus (g/kg) | 5.00 | 5.00 |
| Lysine (g/kg) | 11.6 | 11.6 |
| Methionine (g/kg) | 5.20 | 5.20 |

*[a] Supplied per kg diet, vitamin A, 1900 IU; vitamin, $D_3$ 1300 IU; vitamin E, 10000 mg; vitamin $K_3$, 1000 mg; vitamin B1, 1000 mg; vitamin B2, 5000 mg; vitamin B6, 1500 mg; vitamin B12, 0.046 mg; Biotin, 50 mg; BHT, 10000 mg; Pantothenic acid, 10000 mg; folic acid, 1000 mg; Nicotinic acid, 30000 mg. Supplied Mn 60 mg; Zinc 50 mg; Fe 30 mg; Cu 4 mg; I 3 mg; Selenium 0.1 mg; Co 0.1 mg.*

### *2.3. Carcass criteria and internal organs*

The birds in each treatment were processed after 35 days to assess carcass criteria and internal organs. Individually weighed birds were sacrificed in a humane manner, left to bleed, and then plucked. After the neck, head, viscera, shanks, spleen, digestive tract, heart, gizzard, and belly fat were removed, the rest of the body was weighed. The dressing percentage was determined by dividing the carcass and giblets weight by the live weight. The heart, empty gizzard, spleen, and abdominal fat of each bird were separately weighed and expressed as a percentage of live body weight.

### *2.4. Meat Quality Measurements*

The left side of the breast muscle and left leg from each broiler chicken will be used to measure pH after 24 h (pH24), water holding capacity (WHC), thawing loss, and cooking loss. The pH24 of the collected breast muscles will be recorded by using pH-meter 24 h post slaughtering. The low-speed centrifugation method was conducted to estimate WHC of breast muscles, with a little modification (Honikel *et al.*, 1994). Briefly, about 10 g of intact breast muscle was placed and centrifuged in falcon tube containing glass beads at 10,000 × g and 5 °C for 20 min, then the precipitated meat was instantly removed, dried with filter paper, and reweighed again. The WHC was calculated as the percentage of loss in muscle samples weight after centrifugation (Honikel *et al.*, 1998). Regarding thawing loss, the breast fillet was trimmed, wiped dry, then weighed (initial weight) and stored at –18 °C. After one week, the frozen breast fillets were thawed at 5 °C for 24 h and the final weight was calculated. The percentage of the difference between initial and final weight was the value of thawing loss (Honikel *et al.*, 1998). Cooking loss was determined as described earlier. Briefly, the muscle fillets were separately placed in thin-walled thermotolerant plastic bags in a water bath until core temperature reached 70 °C, after which they were cooled to 5 °C in crushed ice, and reweighed again to calculate the cooking loss.

### *2.5. Statistical analysis*

The general linear model (GLM) approach of Statistical Analysis System (SAS 2005, Institute, Inc., Cary, NC, USA) software was used to analyze all data. To compare means, Duncan's multiple range test was utilized. Replicate pens were the experimental units for all analyses. The significance level was set at $P \leq 0.05$.

## 3. Results

### *3.1. Growth performance*

The Effects of selenium sources on growth performance of broilers are shown in Table 2. There was no mortality and the general health status of birds was good throughout the experimental period. Broilers fed the diets supplemented with selenium sources increased body weight and body weight gain and improved (P<0.05) feed conversion ratio than those fed the control diets.

**Table 2**. Effects of selenium sources on feed intake and growth performance of broilers

| Items | Treatments | | | | SEM | P-Value |
|---|---|---|---|---|---|---|
| | Control | Organic-Se | Organic-Se | Organic-Se | | |
| | | | Body Weight, g | | | |
| 1 day | 41.46 | 42.29 | 41.75 | 43.10 | 0.9 | 0.389 |
| 12 days | 357[b] | 405[a] | 390[ab] | 419[a] | 8 | 0.032 |
| 24 days | 1005[b] | 1175[a] | 1178[a] | 1222[a] | 22 | 0.001 |
| 35 days | 1834[c] | 2048[b] | 2031[b] | 2119[a] | 24 | 0.001 |
| | | | Body weight gain, g | | | |
| 1-12 days | 322[b] | 363[a] | 349[ab] | 376[a] | 7 | 0.042 |
| 12-24 days | 647[b] | 770[a] | 787[a] | 803[a] | 18 | 0.001 |
| 24-35 days | 828 | 872 | 852 | 896 | 16 | 0.500 |
| 1-35 days | 1792[c] | 2006[b] | 1989[b] | 2076[a] | 24 | 0.001 |
| | | | Feed intake, g | | | |
| 1-12 days | 541 | 526 | 507 | 522 | 9 | 0.665 |
| 12-24 days | 1125 | 1150 | 1119 | 1140 | 17 | 0.927 |
| 24-35 days | 1453[a] | 1353[ab] | 1270[b] | 1411[a] | 25 | 0.045 |
| 1-35 days | 3119[a] | 3030[ab] | 2897[b] | 3074[a] | 28 | 0.016 |
| | | | Feed conversion ratio | | | |
| 1-12 days | 1.683[a] | 1.451[b] | 1.457[b] | 1.387[b] | 0.033 | 0.008 |
| 12-24 days | 1.736[a] | 1.503[b] | 1.433[b] | 1.419[b] | 0.036 | 0.001 |
| 24-35 days | 1.756[a] | 1.561[b] | 1.493[b] | 1.572[b] | 0.026 | 0.005 |
| 1-35 days | 1.740[a] | 1.510[b] | 1.456[b] | 1.480[b] | 0.025 | 0.001 |

Broilers fed the diets supplemented with Nano-Selenium had higher body weight and body weight gain and lower (P<0.05) feed conversion ratio than those fed the control diets or diets supplemented with selenomethonine and sodium selenite. Overall, Nano-selenium resulted in best performance.

### *3.2. Meat Quality*

The effect of feeding selenium sources on meat quality in broiler chickens is presented in Table 3. Additionally, broilers fed the diets supplemented with different sources of selenium at 0.30 mg/kg had improved meat quality in leg muscle than those fed the control diets.

**Table 3**. Effects of selenium sources on Meat quality of broilers.

| Items | Treatments | | | | SEM | P-Value |
|---|---|---|---|---|---|---|
| | Control | Organic-Se | IN-Organic-Se | Nano-Organic-Se | | |
| | | | Breast meat | | | |
| PH | 5.016 | 5.100 | 5.133 | 5.100 | 0.027 | 0.497 |
| Cooking loss | 29.43[a] | 27.63[ab] | 25.24[b] | 29.74[a] | 0.727 | 0.099 |
| Water capcity | 25.63 | 27.27 | 26.03 | 29.79 | 0.696 | 0.137 |
| | | | Leg meat | | | |
| PH | 5.116 | 5.066 | 5.100 | 5.183 | 0.031 | 0.629 |
| Cooking loss | 35.40[a] | 27.85[b] | 26.25[b] | 25.02[b] | 1.076 | 0.002 |
| Water capcity | 25.55 | 21.94 | 24.74 | 21.44 | 0.837 | 0.224 |

### *3.3. Carcass criteria*

The effect of feeding selenium sources on carcass criteria in broiler chickens is presented in Table 4. Furthermore, broilers fed the diet supplemented with different sources of selenium improved dressing percentage and abdominal compared to control, but no differences (P<0.05) were observed in internal organs among treatments.

**Table 4.** Effects of selenium sources on carcass criteria of broilers.

| Items | Treatments | | | | SEM | P-Value |
|---|---|---|---|---|---|---|
| | Control | Organic-Se | Non-Organic-Se | Nano-Organic-Se | | |
| LBW | 1831[b] | 2023[a] | 2022[a] | 2046[a] | 18.36 | 0.001 |
| Carcass W | 67.12[b] | 70.69[a] | 70.43[a] | 71.74[a] | 0.330 | 0.001 |
| Breast W | 39.56[b] | 41.66[ab] | 41.14[ab] | 43.58[a] | 0.474 | 0.022 |
| Leg W | 31.00 | 31.36 | 30.03 | 29.07 | 0.721 | 0.690 |
| Liver | 2.00 | 1.93 | 2.23 | 2.13 | 0.057 | 0.285 |
| Heart | 0.501 | 0.471 | 0.495 | 0.498 | 0.008 | 0.601 |
| Gizzard | 1.307[ab] | 1.163[b] | 1.434[a] | 1.326[ab] | 0.037 | 0.079 |
| FATS | 541[a] | 526[b] | 507[b] | 522[b] | 0.044 | 0.006 |
| Small W | 2.71[c] | 3.47[ab] | 3.06[bc] | 3.66[a] | 0.102 | 0.002 |
| Small L | 169[b] | 181[a] | 172[b] | 183[a] | 1.361 | 0.001 |
| Cecum W | 0.740[b] | 0.764[b] | 0.941[a] | 0.991[a] | 0.026 | 0.002 |
| Cecum L | 112 | 115 | 114 | 115 | 0.652 | 0.304 |
| Spleen | 0.109 | 0.121 | 0.105 | 0.118 | 0.004 | 0.585 |

## 4. Discussion

Global Reviewing the results of the effects of selenium sources on growth performance of broilers showed a good general health status of birds throughout the experimental period. Broilers fed the diets supplemented with selenium sources increased body weight and body weight gain and improved (P<0.05) feed conversion ratio than those fed the control diets. Broilers fed the diets supplemented with Nano-Selenium had higher body weight and body weight gain and lower ($P<0.05$) feed conversion ratio than those fed the control diets or diets supplemented with selenomethonine and sodium selenite. Overall, Nano-selenium resulted in best performance. These findings are in close agreement with several authors such as Ahmadi *et al.* (2018) indicated significant improvement in weight gain and feed conversion ratio in starter, grower, and whole periods of experiment when diet supplemented by nano-Se. When broiler chicks were fed Se yeast as an organic form of Se or when nano Se was used growth performance was improved, These findings were consistent with many previous studies, including those by Selim *et al.* (2015) observed improvements in growth performance parameters such as body weight (BW), body weight gain (BWG), and feed conversion ratio (FCR). Supplementation of Nano-Se improved growth performance (Zhou and Wang, 2011; Dlouha *et al.*, 2008; Upton *et al.*, 2008; Fu-xiang *et al.*, 2008). Zhou and Wang (2011) clearly indicate that providing Nano-Se supplemented diet, could improve the final BW, DWG and FCR of Guangxi Yellow chickens. Also, Upton *et al*. (2008) reported that broilers given diets supplemented with 0.2 mg/kg of organic Se showed significant increase in the BW as compared with a diet supplemented with inorganic Se and a control diet.

Srimongkol *et al.* (2004) reported that adding the organic form of Se enhanced performance parameters during growing, finishing and overall periods.

Regarding the impact of selenium sources on broiler chickens' meat quality, it was clear that broilers fed the selenium-added diets had superior meat quality in the leg muscle compared to those fed the control diets. Aside from that, the findings are consistent with other research, such as that of Bakhshalinejad *et al.* (2019), who found that employing several sources (SS, SY, SM and NS) at levels of 0.1 and 0.3% Se had no impact on the pH of the breast or thigh meat of broiler chickens. This is in match with Peric *et al*. (2009) who reported that no significant differences between treatments in pH of the breast or thigh meat when adding (0.1, 0.2, 0.3) ppm of organic selenium from (Sel-plex) and inorganic source from sodium selenite. Also, Jamnongtoi *et al*. (2018) indicated that there was no effect of Se source from (organic Zn-L-selenomethionine (Zn-L-SeMet) and inorganic sodium selenite (Na-Se) on broiler in diet on drip loss. Again, Göçmen *et al*. (2016) recorded no significant effect on pH, cook loss (CL) or penetrometer values (PM) for breast and thigh meat in broilers when diet supplemented by various Se sources from organic (Sel –plex 50) and inorganic from (sodium selenite) at different levels (0, 0.15, 0.30 and 0.60). Boiago *et al*. (2014) observed that there was no effect ($P>0.05$) of selenium supplementation on the WHC, CL and pH when used different levels from Se at (0.3 and 0.5 mg kg) in the form of selenomethionine (Se-Met) and sodium selenite (SS). However, Chen *et al*. (2013) showed no significance on broilers in drip loss among the different groups from selenium yeast at levels (0.3 ,0.5, 1.0 and 2.0 mg .kg) all over the entire period. This was in match with Payne *et al*. (2005) who provided that breast meat had no significant drip loss by altering Se level.

In the current study, broilers given a diet supplemented with various sources of selenium had improved abdomen and dressing percentages compared to controls, but there were no differences ($P>0.05$) in internal organs among treatments. These outcomes were in line with those of Bakhshalinejad *et al.* (2019), who did not find any relevance with regard to the yield of carcass, breast, and thigh muscles when diet was supplemented with various Se sources and amounts.Additionally, Ahmadi *et al.* (2018) demonstrated that there were no differences in the weights of the thymus, lungs, kidneys, pancreas, testicles, proventriculus, right and left cecum, and non-edible organs (liver, heart, and gizzard) between the experimental groups. Additionally, they demonstrated that there were no variations in the proportions of breast and drumsticks, abdominal fat, or non-edible organs. Moreover, Jamnongtoi *et al.* (2018) found that employing various sources of Se had no impact on the carcass criterion for broiler chickens). They are also in agreement with Chen *et al.* (2014) and Cai. *et al.* (2012) who found no effect of Se addition (nano-Se or sodium selenite/selenium enriched yeast, respectively) on the weights of bursa of Fabricius, thymus and spleen.

## 5. Conclusion

The results from the present study indicated that supplemental selenium improved the growth performance, physiochemical meat quality and carcass criteria of broilers; and the Nano-selenium was more effective than the Se from selenomethonine and sodium-selenite.

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*All authors are contributed in this research.*

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**Institutional Review Board Statement**

*All Institutional Review Board Statements are confirmed and approved.*

**Data Availability Statement**

*Data presented in this study are available on fair request from the respective author.*

**Ethics Approval and Consent to Participate**

*Not applicable*

**Consent for Publication**

*Not applicable.*

**Conflicts of Interest**

*The authors disclosed no conflict of interest starting from the conduct of the study, data analysis, and writing until the publication of this research work.*

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