

## NANO SELENIUM TREATMENT EFFECTS ON THYROID HORMONES, IMMUNITY AND ANTIOXIDANT STATUS IN RABBITS

EID S.Y.<sup>1</sup>\*, EL-ZAHER H.M.<sup>1</sup>\*, EMARA S.S.<sup>1</sup>\*, FARID O.A.<sup>1</sup>†, MICHAEL M.I.\*

\*Biological Applications Department, Radioisotope Applications Division, Nuclear Research Centre, Atomic Energy Authority, Inshas13759, CAIRO, Egypt.

†Physiology Department, National Organisation for Drug Control and Research (NODCAR), GIZA, Egypt.

**Abstract:** The present study was conducted to compare the effect of Nano-Selenium (Nano-Se) and sodium selenite (SSe) on antioxidant enzyme activity, immunity and thyroid activity of growing New Zealand White (NZW) rabbits. In this study, 72 male rabbits (5 wk old) were divided randomly into 3 groups (24 rabbits each). The first group served as a placebo; in groups 2 and 3, each rabbit was intramuscularly injected once a week with 4 mL solution of Nano-Se or SSe, respectively, for a 2-mo period. The solution was adjusted to provide 30 µg Se/kg/live body weight. Results showed that Nano-Se treatment significantly ( $P<0.0001$ ) increased in superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) more than control, but decreased significantly each of glutathione disulphide (GSSG) and nitric oxide (NO) levels in serum. Likewise, supplementation of SSe increased ( $P<0.0001$ ) GPx activity and significantly decreased both malondialdehyde (MDA) and GSSG levels more than control. Nano-Se significantly enhanced serum IgG and IgM more than SSe and control groups. Serum aspartate aminotransferase increased ( $P<0.0001$ ) due to Nano-Se treatment as compared to control and SSe, although the lowest ( $P<0.0001$ ) activity of alanine aminotransferase was recorded due to SSe supplementation. Nano-Se treatment increased ( $P<0.0001$ ) both T3 and T4 concentrations more than other groups. Furthermore, administration of Nano-Se increased SOD, GPx, GSH, total antioxidant capacity (TAC) and adenosine triphosphate (ATP) in liver tissue of growing rabbits, while it decreased MDA and 8-hydroxy-2'-deoxyguanosine (8-oHdG) levels in liver tissue compared with control. Also, SSe showed an increase ( $P<0.0001$ ) in GSH, and ATP, but significantly decreased TAC and MDA levels compared with control. It can be concluded that Nano-Se supplementation significantly enhanced the activity of antioxidant enzymes in both serum and liver tissues, with a greater positive influence on immunoglobulin production and thyroid activity in growing NZW rabbits than SSe.

**Key Words:** Nano-selenium, sodium selenite, thyroid activity, antioxidant enzymes, rabbits.

## INTRODUCTION

Selenium is an essential trace element important for many physiological processes, especially for immune and antioxidant defence functions, as well as metabolism of thyroid hormones. The most important action of selenium biological functions comes from several specific selenoproteins, some of which are involved in thyroid hormone metabolism, while others play an antioxidant defence role (Ganong, 2001).

Selenium is required for the conversion of thyroxin (T4) into the more active triiodothyronine (T3) via the enzyme type 4 deiodinase (Awadeh *et al.*, 1998). Additionally, selenoperoxidases and thioredoxin-reductase protect the thyroid gland from peroxides produced during hormone synthesis (Arthur and Beckett, 1999). Selenium is also necessary for immune function regulation and has a vital role in non-specific immune response (Dercksen *et al.*, 2007; Köhrle and Gärtner, 2009). The weakened immune system is a result of Se deficiency (Efraimidis and Wiersinga, 2014).

**Correspondence:** Sherif Y. Eid, [sherifyousif00@gmail.com](mailto:sherifyousif00@gmail.com). Received January 2019 - Accepted May 2019.  
<https://doi.org/10.4995/wrs.2019.11251>

Nano-elemental Se (Nano-Se) possesses comparable efficiency with other Se sources (Zhang *et al.*, 2008) and has more activity in up-regulating selenoenzymes than selenite and selenomethionine, while exhibiting a dramatic decrease in acute toxicity (Qin *et al.*, 2014). Nano-Se has attracted more attention because of its high bioavailability, high catalytic efficiency, strong adsorbing ability and low toxicity compared with selenite in rats (Jia *et al.*, 2005), mice (Wang *et al.*, 2007) and goats (Shi *et al.*, 2011).

Few studies showed that Nano-selenium intake could enhance the antioxidant activity of the animals (Zhu *et al.*, 2010), while Nasirpour *et al.* (2017) concluded that nano-selenium supplementation ameliorates the negative effects of oxidative stress on liver and positively influences immunoglobulin production.

However, the effect of Nano-Se on thyroid gland activities in growing rabbits has had little attention from scientists and the studies on Nano-Se supplementation are still scarce and the results rather discordant. Thus, the purpose of this experiment is to study the effects of supplementary Nano-Se on thyroid gland and antioxidant enzyme activities and immunoglobulin levels in serum and liver of growing rabbits in comparison with selenium as sodium selenite (SSe).

## MATERIALS AND METHODS

This study was carried out in the farm belonging to the rabbit's research and breeding Project, Nuclear Research Centre, Egyptian Atomic Energy Authority, Inshas, Egypt.

### Ethical approval

The current work was approved by the EAEA Committee, No. (180) 25/11/2018. Rabbits were handled according to EC Directive 86/609/EEC for animal experiments.

### Experimental design

A total of 72 NZW male rabbits (5 wk old) with mean body weight around 630.4±3.7 g were used in this experiment. The rabbits were randomly divided into 3 groups (24 in each); group one was injected (IM) once a week with 4 mL double distilled water as a control. Groups 2 and 3 were injected by the same route with a solution of Nano-Se or SSe, respectively, adjusted to provide 30 µg Se/kg/live body weight. The experimental period lasted 2 mo.

### Animal feeding

Ingredients and chemical analyses of the basal diet are presented in Table 1 following NRC (1977) recommendations. The samples of the experimental basal diet were ground in a hammer mill fitted with a 1 mm pore size screen and analysed in triplicate for their content in dry matter, ash, crude protein (N×6.25), crude fibre and ether extract according to AOAC (2000). Nitrogen-free extract was calculated by differences.

Shanghai Stone Nano-Technology Port Co. Ltd., China provided NANO-Se CAPSULE®. This product is based on a liquid Nano-Se which is considered the main health care supplement ingredient. The sizes of elemental Se particles ranged from 60 to 80 nm, in the form of orange

**Table 1:** Ingredients and the chemical compositions of the experimental ration.

Ingredients	(%)
Clover hay	38.00
Wheat bran	24.00
Yellow corn	15.00
Soybean meal (44% CP)	16.00
Molasses	4.00
NaCl	1.00
Sodium bicarbonate	0.10
Di-Calcium phosphate	1.60
Vitamins and Minerals premix <sup>1</sup>	0.30
Total	100
Calculated composition on dry matter basis (%) <sup>2</sup>	
Crude protein (CP)	17.20
Ether extract	2.52
Crude fibre	14.55
Digestible energy (MJ/Kg diet)	10.47
Calcium	1.00
Phosphorus	0.85
Starch	50.58
Lysine	0.84
Methionine	0.29

<sup>1</sup>Each 1 kg of vitamins and minerals premix contained: Vitamin A, 10 000 IU, Vitamin D3, 1800 IU; Vitamin E, 15 mg; vitamin K3, 4.5 mg; Vitamin B1, 0.5 mg; Vitamin B2, 4 mg; Vitamin B12, 0.001 mg; Folic acid, 0.1 mg; Pantothenic acid, 7 mg; Nicotinic acid, 20 mg; I, 1 mg; Mn, 60 mg; Cu, 5.5 mg, Zn, 75 mg; Fe, 40 mg; Co, 0.3 mg; Se, 0.1 mg; Robenidine, 52.8 mg.

<sup>2</sup>According to NRC (1977).

powder-coated capsules, each containing 0.225 g of powder including Nano-Se (45 µg). The calculated dose of treatments, according to live body weight, was dissolved in double distilled water using a magnetic stirrer overnight, then intramuscularly injected.

### Animal management

The rabbitry building was naturally ventilated through wired windows and provided with electronic controlled sided exhaustion fans. Rabbits were individually housed in galvanised wired battery cages (50×55×39 cm); each cage had its feeder and automatic nipple drinker. Feed and water were offered *ad libitum*. All animals were kept under the same managerial, hygienic and environmental conditions and were maintained and treated in adherence to the accepted standards of the humane treatment of animals. The rabbits in all experimental groups were vaccinated with clostridial enterotoxaemia bloat, bacterial and viral immunisation (Veterinary Research and Vaccines, Research Institute, Cairo, Egypt).

### Blood sampling and analysis

After 8 wk from the starting date, at 13 wk of age, blood samples were collected from the marginal ear vein of the rabbits into clean tubes in each group. The blood samples were left to clot, then centrifuged (4000 rpm) for 10 min; the clear serum was collected and stored at -70°C until the subsequent biochemical and hormonal analyses. Samples of 8 animals from each group were also slaughtered at the end of the experimental period for organ analysis and statistical performance; liver and thyroid organs were removed directly and preserved at -70°C until analysis.

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined using commercial kits manufactured by the Bio-Diagnostic Company, Egypt.

The activities of glutathione peroxidase (GPx), reduced glutathione (GSH), oxidised glutathione (GSSG), superoxide dismutase (SOD) and malondialdehyde (MDA) (Papadoyannis *et al.*, 1999; Paglia and Valentine, 1967; Goldberg and Spooner, 1983; Habig *et al.*, 1974) were estimated according to the manufacturer's instructions using kits from Biodiagnostic company (Dokki, Giza, Egypt). Nitric oxide was determined as the sum of nitrite and nitrate according to the method of Everett *et al.*, (1995). Serum IgG and IgM were measured using the rat IgG and IgM ELISA kit (Life Diagnostics Inc., PA, USA).

The radioimmunoassay (RIA) technique was applied for serum triiodothyronine (T<sub>3</sub>) and thyroxine (T<sub>4</sub>) estimation using antibody-coated tubes kit purchased from DIA source ImmunoAssays S.A. Belgium. The assay was carried out in the Laboratory of Biological Applications Department, Atomic Energy Authority, using a Gamma Counter.

Adenosine triphosphate (ATP) in liver tissues was carried out by high performance liquid chromatography (HPLC) (Liu *et al.*, 2006) and reduced glutathione (GSH) contents were estimated by the HPLC method developed by Jayatilleke and Shaw, 1993 for the measurement of oxidised and reduced glutathione in biological samples. Furthermore, the TAC was estimated in these tissues by the colorimetric method (Koracevic *et al.*, 2001). Antioxidant enzymes such as SOD (Sun *et al.*, 1988) and glutathione peroxidase activity (Gpx) (Lawrence and Burk, 1976) were measured in liver tissue. MDA was estimated according to the method of Karatepe, (2004) using HPLC with a UV detector. The separation of 8-OHdG was performed with an Agilent HP 1200 series HPLC apparatus (USA) by the method of Lodovici

Table 2: Effect of Nano-Se and sodium selenite on thyroid hormones level and liver enzymes activity of growing rabbits.

Treatment	T3 (nmol/L)	T4 (nmol/L)	AST (U / mL)	ALT (U / mL)
Control	1.26 <sup>a</sup> ±0.03	33.09 <sup>a</sup> ± 0.42	18.96 <sup>a</sup> ±0.26	24.95 <sup>c</sup> ±0.20
SSe	1.43 <sup>a</sup> ± 0.07	39.35 <sup>b</sup> ±1.15	19.24 <sup>a</sup> ± 0.19	18.46 <sup>a</sup> ±0.17
Nano-Se	2.94 <sup>b</sup> ± 0.11	58.56 <sup>c</sup> ± 1.10	23.34 <sup>b</sup> ± 0.44	24.14 <sup>b</sup> ± 0.22
F-value	147.60	195.02	60.97	325.80
P-value	0.0001	0.0001	0.0001	0.0001

<sup>a, b, c</sup> Means within columns with different superscript are significantly different at P<0.05.

T3: triiodothyronine, T4: thyroxine, AST: aspartate aminotransferase, ALT: alanine aminotransferase, SSe: sodium selenite, Nano-Se: Nano-selenium.

**Table 3:** Effect of treatments on serum antioxidant activity of growing rabbits.

Treatment	SOD (U/mL)	MDA (nmol/mL)	GSH ( $\mu$ mol/mL)	GPx (U/L)	GSSG ( $\mu$ mol/mL)
Control	27.67 $\pm$ 0.42 <sup>a</sup>	1.46 $\pm$ 0.04 <sup>b</sup>	9.19 $\pm$ 0.38 <sup>a</sup>	25.75 $\pm$ 0.6 <sup>a</sup>	0.51 $\pm$ 0.03 <sup>b</sup>
SSe	26.75 $\pm$ 0.49 <sup>a</sup>	1.22 $\pm$ 0.04 <sup>a</sup>	8.92 $\pm$ 0.23 <sup>a</sup>	28.07 $\pm$ 0.65 <sup>b</sup>	0.38 $\pm$ 0.04 <sup>a</sup>
Nano-Se	32.50 $\pm$ 0.83 <sup>b</sup>	1.54 $\pm$ 0.07 <sup>b</sup>	11.47 $\pm$ 0.56 <sup>b</sup>	31.65 $\pm$ 0.85 <sup>c</sup>	0.33 $\pm$ 0.02 <sup>a</sup>
F-value	25.5	9.68	11.33	17.55	10.16
P-value	0.0001	0.0001	0.0001	0.0001	0.0001

<sup>a, b, c</sup> Means within columns with different superscript are significantly different at  $P < 0.05$ .

SOD: superoxide dismutase, MDA: malondialdehyde, GSH: reduced glutathione, GPx: glutathione peroxidase, GSSG: glutathione disulphide, SSe: sodium selenite, Nano-Se: Nano-selenium.

*et al.*, (1997), modified by Abu-Qare and Abou-Donia, (2000). Selenium determinations in serum and tissues were performed following the method of Stadlober *et al.* 2001.

### Statistical analysis

Statistical data were analysed using the general linear model (GLM) SAS procedure (2000) and the significant differences between means were verified by Duncan Multiple tests.

## RESULTS

Nano-Se and sodium selenite effects on thyroid hormone levels and liver enzyme activity of growing rabbits are illustrated in Table 2. Nano-Se increased ( $P < 0.0001$ ) both T3 and T4 concentrations more than other groups, with the highest mean values of 2.94 and 58.56 nmol/L, respectively. Sodium selenite increased ( $P < 0.0001$ ) only T4 (39.35 nmol/L) than control. Serum AST increased ( $P < 0.0001$ ) due to Nano-Se treatment (23.34 U/mL) as compared to control and SSe, although both Nano-Se and SSe decreased ( $P < 0.0001$ ) ALT. The lowest value was 18.46 U/mL, due to SSe supplementation (Table 2).

The effect of treatments on serum antioxidant activities of SOD, MDA, GPx, GSH, GSSG and NO is shown in Tables 3 and 4, as well as the immunity profile of growing rabbits. Nano-Se administration increased ( $P < 0.0001$ ) SOD, GPx and GSH more than control, but significantly decreased GSSG and NO levels. There was no difference ( $P > 0.05$ ) between Nano-Se and control for MDA level. Supplementation of growing rabbits with SSe increased ( $P < 0.0001$ ) GPx activity and significantly decreased both MDA and GSSG levels compared to control. On the other hand, Nano-Se enhanced ( $P < 0.0001$ ) serum contents of IgG and IgM more than SSe and control groups, with the highest mean value of 35.30 ng/mL for IgM, where SSe increased ( $P < 0.0001$ ) only IgM (27.10 ng/mL) more than control (Table 4).

Nano-Se and SSe effects on SOD, GPx, GSH, MDA, TAC, ATP and 8-oHdG activities in liver tissue are presented in Tables 5 and 6. Administration of Nano-Se increased SOD, GPx, GSH, TAC and ATP in liver tissue of growing rabbits with the highest value of 527.52 U/g tissue,  $P < 0.0001$  for TAC compared to control (429.47U/g tissue); while it

**Table 4:** Effect of treatments on serum antioxidant activity and immunity of growing rabbits.

Treatment	NO ( $\mu$ mol/mL)	IgG (ng/mL)	IgM (ng/mL)
Control	0.47 $\pm$ 0.02 <sup>b</sup>	26.34 $\pm$ 0.54 <sup>a</sup>	23.88 $\pm$ 0.27 <sup>a</sup>
SSe	0.44 $\pm$ 0.02 <sup>b</sup>	27.65 $\pm$ 0.58 <sup>a</sup>	27.10 $\pm$ 0.58 <sup>b</sup>
Nano-Se	0.39 $\pm$ 0.01 <sup>a</sup>	33.77 $\pm$ 0.61 <sup>b</sup>	35.30 $\pm$ 0.72 <sup>c</sup>
F-value	4.23	47.24	112.19
P-value	0.019	0.0001	0.0001

<sup>a, b, c</sup> Means within columns with different superscript are significantly different at  $P < 0.05$ .

NO: nitric oxide, IgG: immunoglobulin G, IgM: immunoglobulin M, SSe: sodium selenite, Nano-Se: Nano-selenium.

**Table 5:** Effect of treatments on liver antioxidant activity of growing rabbits.

Treatment	ATP (µg/g tissue)	SOD (U/g tissue)	GSH (µmol/g tissue)	GPx (U/g tissue)
Control	26.94±0.8 <sup>a</sup>	57.74±1.4 <sup>a</sup>	15.48±0.52 <sup>a</sup>	26.92±0.55 <sup>a</sup>
SSe	29.40±0.23 <sup>b</sup>	60.26±1.3 <sup>a</sup>	18.48±0.84 <sup>b</sup>	26.63±0.32 <sup>a</sup>
Nano-Se	33.24±0.81 <sup>c</sup>	73.24±1.5 <sup>b</sup>	20.86±0.99 <sup>c</sup>	32.93±0.76 <sup>b</sup>
F-value	22.58	52.29	11.21	38.45
P-value	0.0001	0.0001	0.0001	0.0001

<sup>a, b, c</sup> Means within columns with different superscript are significantly different at  $P<0.05$ .

ATP adenosine triphosphate, SOD superoxide dismutase, GSH reduced glutathione, GPx glutathione peroxidase. (N=8)

markedly decreased MDA and 8-oHdG levels in liver tissue compared with other groups. Sodium selenite showed an increase ( $P<0.0001$ ) in GSH and ATP, but significantly decreased TAC, MDA and 8-oHdG levels as compared to control. SSe did not differ ( $P>0.05$ ) from control for the other parameters (Tables 5 and 6).

Results of Table 7 revealed higher ( $P<0.001$ ) Se concentrations in serum, liver and thyroid gland tissues for the Nano-Se treatment group than for SSe.

## DISCUSSION

The current results showed a greater increase in serum T3 and T4 of rabbits supplemented with Nano-Se than with SSe and in control. These results are in agreement with the findings of Rezaeian-Tabrizi *et al.* (2017), who reported a greater increase in T4 and T3 in non-stressed animals given Nano-Se than in control, and Changguang *et al.* (2013), who showed that piglets' serum T3 level of Nano-Se treatments significantly increased compared to the control group, and significantly higher than sodium selenite groups. These results may be due to the effective role of the selenium element in thyroid gland function. The iodothyronine 5'-deiodinases - which are necessary for the production of active T3 hormone function- are selenoenzymes, and the thyroid gland has high selenium concentrations, mostly for the function of deiodinases enzymes which catalyse the conversion of T4 to T3 (Köhrle *et al.*, 2007).

The present study reported that Nano-Se supplementation increased serum AST activity compared to control and SSe, although the lowest activity of ALT was due to SSe supplementation. This agrees with the findings of Mohapatra *et al.* (2014), who reported a linear and quadratic increase in serum AST levels with Nano-Se increment in the diet. On the contrary, Qin *et al.* (2016) found that Nano-Se and SSe supplementation increased ALT level more than control, where Nano-Se decreased AST more than SSe and control groups. Moreover, Kumar *et al.* (2008) indicated that SSe had no effect on serum AST and ALT activities. These enzymes are cytoplasmic enzymes released into the blood after hepatic cellular damage. Under oxidative stress conditions, ROS (reactive oxygen species) are generated and hydroxyl radicals increase peroxidation of fatty acids, leading to cell membrane damage and rupture. Nano-Se injection had no effect ( $P<0.0001$ ) on these enzymes except AST. This may be due to fact that AST is not a liver-specific enzyme; it is

**Table 6:** Effect of treatments on liver antioxidant activity of growing rabbits.

Treatment	TAC (U/g tissue)	MDA (nmol/g tissue)	8-OHdG (Pg/g tissue)
Control	429.47±4.1 <sup>b</sup>	29.95±0.18 <sup>c</sup>	307.83±4.2 <sup>c</sup>
SSe	411.33±4.0 <sup>a</sup>	27.25±0.23 <sup>b</sup>	250.33±5.2 <sup>b</sup>
Nano-Se	527.25±3.2 <sup>c</sup>	17.35±0.14 <sup>a</sup>	177.0±2.7 <sup>a</sup>
F-value	266.28	1234.6	243.08
P-value	0.0001	0.0001	0.0001

<sup>a, b, c</sup> Means within columns with different superscript are significantly different at  $P<0.05$ .

TAC: total antioxidant capacity, MD: malondialdehyde, 8-OHdG: 8-hydroxy-2'-deoxyguanosine. (N=8). SSe: sodium selenite, Nano-Se: Nano-selenium.

**Table 7:** Selenium concentrations in different tissue of growing rabbits.

Treatment	Serum (ng Se/mL)	Liver ( ng Se/g tissue)	Thyroid (ng Se g tissue)
SSe	25.41±0.58	15.19±0.09	2.89±0.08
Nano-Se	31.49±0.83	19.37±0.35	3.74±0.04
F-value	35.84	131.658	90.256
P-Value	0.001	0.001	0.001

SSe: sodium selenite, Nano-Se: Nano-selenium.

also excreted from the cardiac and skeletal muscles and Nano-Se may have another effect on these muscles, as well as the current determinations achieved in blood stream, but not liver tissue.

The result of the present study showed that Nano-Se supplementation increased activities of SOD, GPx, GSH in serum and liver tissue of growing rabbits, as well as liver TAC. It also decreased ( $P<0.01$ ) serum GSSG, NO levels and MDA and 8-OHdG in liver tissue. In one line, Nano-Se increased plasma GPx, SOD, TAC and decreased MDA of non-stressed rats more than in control (Razeian-Tabrizi *et al.*, 2017). In addition, Shi *et al.* (2010) and Hao *et al.* (2014) reported an increase of GPx and SOD in tissues of animal groups fed Se, as well as the lowest MDA level. The results for SSe are on the same side as serum GPx, liver tissue GSH, TAC increase and a decrease of GSSG and MDA.

Selenium is necessary for the stability and function of GPx and SOD, and GPx enzyme consumes reduced GSH for the hydrogen peroxide reduction to water and alcohol (Holmgren *et al.*, 2005). Furthermore, both Nano-Se and sodium selenite supplementation acts on liver GPx activity improvement and the decrease of MDA contents in rabbits, with a greater effect for nano-selenium than sodium selenite in increasing liver antioxidant activity in rabbits (Qin *et al.*, 2016; Nasirpour *et al.*, 2017).

The higher antioxidative activity of Nano-Se is obvious from the observed decline in hepatic SOD activity. Moreover, Nano-Se exhibits stronger inhibitory effects than selenite and simultaneously increases the hepatic GSH concentrations, while no clear alteration of the GSH levels was observed with selenite treatment, so that Nano-Se has a more pronounced effect than sodium selenite (Zhang *et al.*, 2008).

Likewise, Huang *et al.* (2003) showed that Se nanoparticles have significant effects on scavenging of the free radicals and of DNA protection against oxidation, as selenium as a functional part of GSH-Px protects the neutrophils and other blood components against peroxidative damage (Bickhardt *et al.*, 1999).

The formation of 8-OHdG, as a product of protein oxidation, is considered a critical biomarker of oxygen-related lesions of DNA and cellular oxidation stress (Kasai, 2002). The current data show a more significant decrease in liver 8-OHdG due to Nano-Se treatment than in other groups, indicating that rabbits which received Nano-Se were not under oxidative status.

The current results are in opposition to those of Abd-Allah and Hashem, (2015), who recorded no differences in NO level due to Nano-Se, although ours were significantly low ( $P<0.019$ ) as compared to control (Table 4). But NO results of SSe are in harmony with the same authors. In a study by Marin-Guzman *et al.* (2000) and Shi *et al.* (2010), the results revealed an increase in ATP concentration in tissue of boar and boar goats fed selenium, which concurs with the current ATP results (Table 5).

From the previous result of our study, an improved antioxidant system of growing rabbits due to Nano-Se administration may also enhance immune system function, which is extremely important at this point in their physiological development. This result is in harmony with those of Changguang *et al.* (2013), who investigated Nano-Se and SSe effects on immunity in piglets and found greater enhancement in serum IgM levels than in control for both Nano-Se and SSe groups. Likewise, the results showed a significant increase in IgG of Nano-Se but not SSe, which are in correspondence with the IgG and IgM results in the current study, whereas Nano-Se increased IgG and IgM, and SSe was higher in IgM more than control. As shown in Table 7, Nano-Se treatment increased Se retention in serum as well as liver and thyroid tissues more than SSe, which is consistent with previous results of Mohapatra *et al.* (2014) and Shi *et al.* (2011). Selenium nanoparticles have higher bioavailability compared to sodium selenite, as proved by

Mohapatra *et al.* (2014) and Zhou and Wang, (2011). This may be due more to the differences in lipophilic properties and metabolic pathways of nanoparticles than normal size of sodium selenite.

Selenium has played a role as an additive factor in increasing lymphocyte reproduction. In addition, it was speculated that selenium supplementation enhances both cell-mediated and humoral immune responses (Sadeghian *et al.*, 2012 and Montgomery *et al.*, 2012). Moreover, Kumar *et al.* (2008) indicated that Se could increase the immune system's capacity to protect cells from free radical injuries.

The different physiological effects of Nano-Se and sodium selenite may be attributed to the different absorption process and metabolic pathways. It has been reported 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, while nanoparticles show new transport and uptake characteristics and exhibit higher absorption efficiencies (Liao *et al.*, 2010). So, selenium nanoparticles also show high biological activity and good absorptive ability due to the interaction between the nanoparticles and  $-NH_2$ ,  $C=O$ ,  $-COO$ , and  $-C-N$  groups of proteins (Zhang *et al.*, 2001).

From our results, it could be concluded that Nano-Se is more effective than sodium selenite in increasing different biochemical parameters, serum and liver antioxidant activities, and immune system with thyroid gland activity in growing NZW rabbits.

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