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ORIGINAL ARTICLE

Dietary supplementation of nano-selenium improves reproductive performance, sexual behavior and deposition of selenium in the testis and ovary of Japanese quail

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ABSTRACT

Objective: Selenium (Se), as the form of selenite, is commonly supplemented in poultry diet, which has low bioavailability and high toxicity. Here, we compared the effects of the supplementation of the diet with Se nanoparticles (nano-Se) on the growth, sexual behavior, and reproductive performance (gonad size, sperm quality traits, and plasma testosterone levels for males and egg production for females) of Japanese quail (*Coturnix coturnix japonica*).

Materials and Methods: Quail chicks (n = 300) aging 14 days were divided into three groups: Group 1 (basal diet and Se at 0.2 mg/kg ration), Group 2 (basal diet and nano-Se at 0.2 mg/kg ration), and Group 3 (basal diet and nano-Se at 0.1 mg/kg ration). Several parameters relating to body weight and egg were measured. Sexual behaviors of the birds were observed by continuous visual scanning. The sperm viability, sperm morphology, and concentration of spermatozoa were determined after staining and microscopic examination. The plasma testosterone levels were determined by indirect enzyme immunoassay assay. The Se concentrations in the testicular, ovarian, and ration samples were measured by flame emission atomic absorption spectrophotometer.

Results: The type or concentration of nano-Se administration had no impact on body weight, feed efficiency, egg production, or egg weight. However, the total feed intake throughout the experiment was reduced in Group 2 at 0.2 mg/kg. Nano-Se supplementation significantly increased the sexual behavior. In general, the deposition of Se in the testicular and ovarian tissues increased with increasing supplement concentration. At the same supplement concentration, the tissue deposition of nano-Se was more enhanced than that of inorganic Se. Nano-Se supplementation improved the testicular functions by enhancing plasma testosterone level and sperm quality traits (sperm count, motility, and viability). This improvement was found more prominent with the lower supplement concentration (when comparing 0.1 *vs.* 0.2 mg/kg diet).

Conclusion: It is concluded that the use of nano-Se (at 0.1 mg/kg) in the ration of Japanese quail improves several reproductive performance parameters.

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Introduction

Selenium (Se) is an essential trace mineral, which is crucial for some physiological functions, including growth, fertility, immunity, hormone metabolism, and protection against oxidative stress [1,2]. Furthermore, Se has essential roles in metabolism in animals through its roles in the active site of glutathione peroxidase (GSH-Px), catalase, and superoxide dismutase [3]. Besides, Se defends the cells

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from damage triggered by harmful free radicals and lipoperoxidases [4]. Due to its beneficial effects, Se supplementation in the diet is a common practice nowadays [5–9]. The supplementation is done mostly using the inorganic forms of sodium selenite or sodium selenate, which have low bioavailability and high toxicity [5].

Se supplementation in the form of Se-nanoparticles (Nano-Se) is much safer than using inorganic or organic Se; hence, it alleviates the problems of high toxicity and low bioavailability [4,6]. Nano-Se administration (at 0.3 mg/kg diet) for chicks until 6 weeks of age improved traits such as body weight and immune status [3]. Zhou and Wang [7] reported that feeding of chicken at 0.3 mg/kg diet improved final body weight, the daily weight gain, and the ratio of feed conversion. Leeson et al. [10] showed an improvement in body weight with enhancing Se levels. Xia et al. [11] reported that chicken receiving nano-Se (at 0.4–1.0 mg/kg) resulted in better performance than chicken receiving sodium selenite. Se supplementation of hen diet in the form of Se yeast increased the hatchability percentage [12].

In chickens, the insufficiency of Se is associated with the loss of body weight [13]. Moreover, Se deficiency is associated with reproductive disorders [14]. According to Shi et al. [15], Se deficiency increases abnormal spermatozoal mitochondria. On the contrary, the supplementation of the diet with nano-Se increases the Se level of the testes. Furthermore, as an outcome of Se administration, the activity of GSH-Px in the testicular tissue and semen increases. Thus, Se is essential for many physiological and biochemical procedures in animals, including those related to reproduction [1].

Organic Se has the most prolonged half-life (i.e., 12–13 days) in the muscle, brain, and lungs of chicken. The shortest half-life of about 4 days was documented in the liver, kidneys, and pancreas of chicken [5]. Humans could acquire Se from residues in chicken meat. The recommended daily Se consumption is 55 µg/day for adult humans. However, the maximum harmless dietary Se intake is high (at 400 µg/day) [16]. However, early toxicity can occur after a dietary intake of 300 µg/day, resulting in endocrine disorders and other harmful effects, including hepatotoxicity, gastrointestinal disturbance, dermatitis, and nail and hair loss [4].

Sexual behavior in poultry is related to the cock which displays numerous courtship behaviors such as waltzing, wing flapping, and tidbitting. These display several purposes and lead to mating and ejaculation of semen and deposition in the female cloaca [17]. Several hormones control sexual maturity, semen production, and sexual behavior in male broiler breeders. Testosterone is the primary sex hormone in the avian testis. Changes in the secretion of this hormone are likely to correlate with testicular activity and sexual behavior [18]. Intramuscular injection of sodium selenite increases serum testosterone levels in male broilers [19]. In addition, the supplementation of feed with Se and vitamin E enhances semen quality manifested by increased sperm viability and reduced morphologically abnormal spermatozoa in broiler breeders and quails [8,9,20].

There is good evidence, mostly from domestic chicken, and Se has physiological importance and that nano-Se has improved bioavailability; however, there is a lack of data on the impacts of nano-Se in Japanese quail. The objectives of this study were to compare the effects of supplementing the diet with nano-Se (instead of inorganic Se) on growth, sexual behavior, and reproductive performance of Japanese quail (*Coturnix coturnix japonica*).

Materials and Methods

Ethical approval

The research was conducted at the Animal Husbandry and Animal Wealth Development Department, Faculty of Veterinary Medicine, Alexandria University, Egypt. We followed the guidelines set by the National Research Council (NRC) for the use and care of poultry and animals for research. The local committee approved the protocols of the experiment for Ethics for the Care and Use of Laboratory Animals of Alexandria University, Egypt (Permit #2020/013/57).

Animals and management

A total of 300 chick (14 days old) Japanese quails (*Coturnix coturnix japonica*) were acquired from a commercial farm. The birds were allocated into three groups, and each group contained four replicates. Each replicate comprised 25 birds. The birds of the Group 1 were provided with a basal diet supplemented with Se at 0.2 mg/kg diet. The birds of the Group 2 were fed with a basal diet supplemented with nano-Se at 0.2 mg/kg diet. Group 3 was given a basal diet supplemented with nano-Se at 0.1 mg/kg diet. Sodium selenite was obtained from El-Gomhoria Pharmaceutical Chemicals Company, Egypt, whereas nano-Se was collected from NanoShell, Wilmington, Delaware, USA. The chicks were fed a balanced ration prepared according to the NRC [21] recommendations (Table 1). The chicks were allowed free access to water. The lighting program was continuous light till the laying period; the duration was 16 h light and 8 h of darkness.

Chemical analysis of the ration

Moisture and ash levels were determined according to the methods described by the Association of Official Agricultural Chemists [22]. The Kjeldahl method was used

to determine crude protein [23], and ether extract was estimated following the procedures described by Bligh and Dyer [24] and Hanson and Olly [25].

Variables evaluated

Body weight: Body weight was recorded once a week until the 6th week of age.

Body weight gain: Quail body weight gain (expressed in grams) was calculated as the difference between the initial and final weights.

Table 1. Composition and nutrient contents of the basal diet.

Ingredients	Growing (2–6 week)	Laying (7–9 week)
Corn (7.8% CP)	52.3	53.7
Soybean meal (42.9% CP)	36	28.5
Gluten (59.2% CP)	7.7	5.12
Oil	1	1.1
Limestone	1.65	6.1
Monocalcium phosphate(MCP)	0.55	0.7
Wheat bran	0	4
Lysine	0.05	0.05
Methionine	0.06	0.08
Threonine	0.04	0
Choline	0.05	0
Mycotoxin adsorbent	0.05	0.05
Salt	0.25	0.3
Vitamin premix[a]	0.15	0.15
Mineral premix[b]	0.15	0.15
Total	100	100
Chemical analysis		
Moisture%	13.1	11.8
Crude protein%	24.1	20.2
Ether extract%	4.4	4.1
Ash%	6.3	14.4
Se mg/ kg	0.25	0.29
Ca[c]	0.8	2.5
P[c]	0.32	0.37
ME Kcal/kg[c]	2,972.5	2,816.72

[a]Vitamin premix provided per kilogram of diet: vitamin A, 10,000 IU; vitamin D3, 2,500 IU; vitamin E, 20 mg; vitamin K3, 2.5 mg; vitamin B1, 2 mg; vitamin B2, 5 mg; niacin, 30 mg; Ca-D-pantothenate, 8 mg; vitamin B6, 3.5 mg; vitamin B12, 0.015 mg; folic acid, 1 mg; D-biotin, 0.025 mg; vitamin C, 50 mg; choline chloride, 300 mg.
[b]Mineral premix provided per kilogram of diet: manganese, 60 mg; zinc, 25 mg for growing and 50 mg for laying; iron, 120 mg for growing and 60 mg for laying; copper, 5 mg; cobalt, 0.15 mg; iodine, 0.3 mg; selenium, 0.2 mg. Each 1 kg mineral premix contain: Mn sulfate (243.9 g), Zn oxide (31.09 g for growing and 62.189 g for laying), iron carbonate (248.96 g for growing and 124.48 g for laying), copper oxide (6.259 g), cobalt oxide (0.21 g), pot iodide (0.39 g), sodium selenite (0.438 g) and carrier (limestone) up to 1 kg.
[c]Calcium, phosphorus and metabolizable energy (ME) were calculated according to NRC [21].

Feed intake: Animals were provided with feeds every morning. The weight of the diet that was offered and consumed was determined weekly. The average weight difference between the provided feed and the remaining part divided by the number of birds in each group per day was used as daily feed intake. From that, the weekly feed intake was calculated.

Feed conversion ratio (FCR) was given as follows:

a. During the growing period: [26]

$$FCR = \frac{\text{Feed intake (gm)/bird/week}}{\text{Body weight gain (gm)/bird/week}}$$

b. During the laying period:

$$FCR = \frac{\text{Feed intake (gm)/bird/week}}{\text{Produced egg mass (g)/bird/period}}$$

Protein efficiency ratio (PER): Protein utilization was expressed by the PER [27].

$$PER = \frac{\text{Body weight gain (g)/bird/week}}{\text{Protein intake (g)/bird/week}}$$

Protein intake (PI) (g)/bird/week:

PI = amount of feed intake gper week * CP% in the feed (24/100)

The efficiency of energy utilization (EEU): [28]

$$EEU = \frac{\text{ME consumed(Kcal)}}{\text{Body weight gain (g)}}$$

ME consumed (Kcal) = amount of feed intake (gm) per week * (2971.1/1000)

Performance index (PI): [28]

$$PI = \frac{\text{Live body weight(g)}}{FCR} * 100$$

Average egg weight (g): individual eggs weighted for 2 successive weeks

Hen day egg production (HDEP):

$$HDEP = \frac{\text{Number of eggs produced}}{\text{Number of live hens}} * 100$$

Egg mass: Grams of egg produced/hen/day: Egg mass = (HDEP * egg weight) / 100)

Sexual behavior

Sexual behaviors were observed for 3 consecutive weeks, starting from the age of sexual maturity (6th week) until the 9th week of age. Behavioral observation for the mating activity was carried out by continuous visual scanning [29]. Birds were observed for 2 h/day on 3 days/week for 3 weeks. Each day was divided into two phases: morning (6 a.m.:12 p.m.) and afternoon (12 p.m.:6 p.m.). The

observation was done 1 h for each period of the day, which meant 1 h in the morning and 1 h in the afternoon with rotation. On the 1st day, the observation was done from 6:7 a.m. to 12:1 p.m.; then, on the second day, 8:9 a.m. to 2:3 p.m., and so on for the rest of the weeks. Each hour of observation was distributed into 5-min intervals of scanning all birds, then starting with a new 5 min until the end of the observation period. We recorded the number of occurrences of the following male behaviors, as outlined by Dunkan and Hockings [29].

1) *Waltzing*: the cock moves around the hen with short shuffling steps and drops the wings farthest from her.
2) *Wing flapping*: Both wings are raised above the level of the back and flapped.
3) *Tidbitting*: Where the male attracts the females by pretending to have found food in the litter.
4) *Rear approach*: Cock grasping the hen's comb or neck feathers with his beak.
5) *Mounting*: The cock steps on the hens back and grips her wing or back feather with his feet, and her comb or neck feather with his beak.
6) *Treading and full copulation*: The cock makes small treading movements with his feet, followed by mounting and cloacal contact. The cock always stops treading and makes a pelvic thrust, and the hen always immediately after the cock dismount gives a characteristic high-intensity feather shack.

Behavioral patterns were recorded by a single individual making observations from the outside of the pens. For the minimization of distraction by the observer, a 5-min habituation period was allowed before commencing each observation. All birds were kept in mixed-sex flock till the age of sexual maturity, and then, they were sexed at a ratio of 1:1.

Sampling

At 6 weeks of age (beginning of sexual maturity in male quails), a random sample of four males and then another four birds of each sex at the end of the experiment were collected and weighed later euthanized by cervical dislocation. By heart puncture, the blood samples were collected and then transported immediately into heparinized tubes. The collected samples were centrifuged for 10 min at 3,000 rpm using a centrifuge (Centabun 23, Germany). The plasma was stored/frozen for testosterone assay. The right and left testes or left ovary were collected from each bird, dabbed dry of fluid, filter paper, and weighed using a digital balance to calculate the gonadosomatic index (GI = testes or ovaries weight/bodyweight × 100) [30]. The spermatozoa were collected from the vas deferens of each male bird for sperm parameters estimation. A sample from the ration of each group was also saved for the analysis of Se level.

Sperm parameters

Sperm samples were loaded on glass slides (37°C). Subsequently, the following parameters were evaluated and recorded at 100× or 400× magnification: mass motility and individual sperm motility. Sperm viability (live/dead) was determined using eosin–nigrosin stain. Sperm morphology was determined using Giemsa stain. Motility, viability, and morphology data were recorded as percentages. The concentration of spermatozoa was determined by using a hemacytometer counting chamber [31].

Plasma testosterone measurement

Using an indirect enzyme immunoassay assay kit (Monobind, 100 North point Drive, Lake Forest, CA), the testosterone level in the plasma was estimated following the methods described by Tietz [32].

Determination of Se level

In a furnace, the testicular, ovarian, and ration samples were ashed and then digested by five parts of concentrated nitric acid and one part of hydrochloric acid for each 1 gm sample for 24 h [33]. Using flame emission atomic absorption spectrophotometer (Model 210 VGP Buck Scientific, USA), the digested samples were filtered and analyzed for Se per protocols of the instrument manufacturer at a wavelength of 196 nm, bandpass of 0.2 nm, and measuring the time of 3 sec. The metal concentration was presented as mg/kg.

Statistical analysis

The statistical analyses of the data were performed using the SAS software [34]. The one-way analysis of variance (ANOVA) was used for the analysis of growth, production, and behavioral data. The two-way ANOVA was used for the analysis of reproductive parameters. GraphPad prism was used for the analysis of data on testosterone and Se levels in testis. The Duncan's test was used when treatment effects were significant. The overall significance level was set at $p < 0.05$. All values were expressed as the mean ± standard error.

Results

Growth performance

The effects of nano-Se supplementation compared to inorganic Se on growth parameters of Japanese quail are shown in Table 2. The replacement of inorganic Se of Group 1 by 0.2 (Group 2) or 0.1 (Group 3) mg/kg of nano-Se did not affect growth performance ($p > 0.05$). Similarly, the body

weight, weight gain, FCR, the PER, EEU, and PI did not differ among groups. However, the total feed intake was significantly reduced ($p < 0.0001$) in nano-Se in Group 2 that also showed lower FCR than other groups ($p > 0.05$). In addition, Group 2 had the lowest PI and ME, followed by Group 3 and then Group 1 ($p < 0.0001$).

Egg production

Egg production parameters for 2 weeks of egg production are shown in Table 3. At 0.1 mg/kg diet (Group 3), there were numerical ($p > 0.05$) increases in egg weight, egg production, egg mass, and better FCR as compared to other groups. On the other hand, Group 2 showed a significant ($p < 0.05$) reduction in the average daily feed intake during the laying period.

Sexual behavior

Sexual behavioral patterns varied significantly among the supplement groups. As shown in Table 4, nano-Se supplementation significantly increased ($p < 0.001$). Correlation among mating behaviors, waltzing, wing flapping, tidbitting, rear approach, mounting, treading, and full copulation was significant ($p < 0.001$). Moreover, birds exhibited significantly higher sexual behavior in the lower dose (at 0.1 mg/kg) than the higher dose (at 0.2 mg/kg) of nano-Se.

Gonadal and sperm parameters

The effects of nano-Se (at 0.2 or 0.1 mg/kg diet) for 6 and 9 weeks on gonadal and sperm parameters of Japanese quails are shown in Table 5. Nano-Se supplementation significantly increased ($p < 0.0001$) testis weight, ovary weight, mass, and individual sperm motility and spermatozoa concentration compared to inorganic Se in Group1 during both the 6th and 9th weeks of age. In addition, there was a numerical reduction in dead and abnormal sperm percentages in nano-Se treatment compared to Group 1. The weight of testes, mass motility, individual motility, and

Table 2. Effect of nano-selenium on the growth performance of Japanese quail.

Growth parameter	Treatment			
	Group 1 inorganic Se (0.2 mg/kg diet)	Group 2 Nano-Se (0.2 mg/kg diet)	Group 3 Nano-Se (0.1 mg/kg diet)	*p*-value
Initial body weight (g) (2 weeks)	54.78 ± 1.13	55.87 ± 0.87	56.55 ± 1.09	NS
Body weight (g) (3 weeks)	104.40 ± 1.30	102.78 ± 0.98	105.96 ± 1.36	NS
Body weight (g) (4 weeks)	160.90 ± 1.79	158.90 ± 1.34	156.21 ± 1.64	NS
Body weight (g) (5 weeks)	203.88 ± 1.97	203.59 ± 1.61	206.30 ± 1.92	NS
Final body weight (g) (6 weeks)	242.19 ± 2.61	242.52 ± 2.44	245.75 ± 2.72	NS
Total gain (g)	187.41 ± 2.80	186.65 ± 2.71	189.20 ± 2.63	NS
Total feed intake (g)	725.10 ± 0.00^{b}	694.99 ± 0.00^{c}	731.59 ± 0.00^{a}	<0.0001
FCR	3.96 ± 0.06	3.80 ± 0.05	3.94 ± 0.05	NS
PI	174.60 ± 0.00^{b}	167.35 ± 0.00^{c}	176.17 ± 0.00^{a}	<0.0001
PER	1.07 ± 0.02	1.12 ± 0.02	1.07 ± 0.01	NS
ME (Kcal)	2154.34 ± 0.00^{b}	2064.88 ± 0.00^{c}	2173.63 ± 0.00^{a}	<0.0001
EEU	11.77 ± 0.19	11.28 ± 0.16	11.70 ± 0.16	NS
PI	6.35 ± 0.16	6.60 ± 0.17	6.44 ± 0.16	NS

The data were presented as mean ± standard error. Means bearing different superscript letters within the same row are significantly different ($p < 0.05$).

Table 3. Effect of nano-selenium on egg production of Japanese quail.

Item	Treatment			
	Group 1 inorganic Se (0.2 mg/kg diet)	Group 2 Nano-Se (0.2 mg/kg diet)	Group 3 Nano-Se(0.1 mg/kg diet)	*p*-value
Egg weight (g)	12.59 ± 0.19	12.30 ± 0.24	12.70 ± 0.10	NS
HDEP	68.96 ± 2.78	67.80 ± 2.40	71.83 ± 4.04	NS
Egg mass	8.70 ± 0.42	8.35 ± 0.35	9.11 ± 0.51	NS
FI (g)/day	33.22 ± 0.09^{ab}	32.96 ± 0.15^{b}	33.35 ± 0.02^{a}	0.03
FCR	3.92 ± 0.17	4.04 ± 0.16	3.88 ± 0.31	NS

The data were presented as mean ± standard error. Means bearing different superscript letters within the same row are significantly different ($p < 0.05$).

Table 4. Effect of nano-selenium on the sexual behavioral patterns of male Japanese quails.

Behavioral patterns	Treatment			
	Group 1 inorganic Se (0.2 mg/kg diet)	Group 2 Nano-Se (0.2 mg/kg diet)	Group 3 Nano-Se(0.1 mg/kg diet)	*p*-value
Waltzing	8.55 ± 0.96^{c}	10.44 ± 1.26^{b}	13.19 ± 2.23^{a}	<0.0001
Wing flapping	70.92 ± 4.75^{c}	81.55 ± 7.33^{b}	88.93 ± 8.72^{a}	<0.0001
Tidbitting	1.18 ± 0.26^{c}	2.19 ± 0.52^{b}	3.73 ± 0.47^{a}	<0.0001
Rear approach	62.42 ± 7.16^{c}	73.42 ± 8.21^{b}	78.84 ± 6.58^{a}	<0.0001
Mounting	51.18 ± 5.47^{c}	59.52 ± 9.34^{b}	66.34 ± 8.34^{a}	<0.0001
Treading and copulation	71.45 ± 6.05^{c}	82.13 ± 5.21^{b}	87.99 ± 7.87^{a}	<0.0001

The data were presented as mean ± standard error. Means bearing different superscript letters within the same row are significantly different ($p < 0.05$).

Table 5. Effect of nano-selenium supplementation on the male reproductive parameters and female ovary index weight of Japanese quail.

Fertility parameters Week	Treatment			
	Group 1 inorganic Se (0.2 mg/kg diet)	Group 2 Nano-Se(0.2 mg/kg diet)	Group 3 Nano-Se(0.1 mg/kg diet)	*p*-value
Testes index weight				
6 weeks	0.83 ± 0.04^{cB}	1.93 ± 0.10^{bB}	2.62 ± 0.08^{aB}	<0.0001
9 weeks	2.09 ± 0.13^{cA}	3.35 ± 0.16^{bA}	4.20 ± 0.13^{aA}	<0.0001
p-value	<.0001			
Interaction	NS			
Mass motility (%)				
6 weeks	45.77 ± 3.41^{cB}	75.89 ± 1.71^{bB}	87.45 ± 1.15^{aB}	<0.0001
9 weeks	72.29 ± 1.36^{cA}	82.52 ± 1.45^{bA}	92.16 ± 1.71^{aA}	<0.0001
p-value	<.0001			
Interaction	<.0001			
Individual motility (%)				
6 weeks	41.89 ± 2.33^{cB}	80.72 ± 2.00^{bB}	89.27 ± 1.74^{aB}	<0.0001
9 weeks	74.42 ± 1.58^{cA}	88.41 ± 1.19^{bA}	95.10 ± 1.03^{aA}	<0.0001
p-value	<.0001			
Interaction	<.0001			
Dead sperms (%)				
6 weeks	51.52 ± 3.31^{aA}	21.68 ± 1.12^{bA}	16.21 ± 1.02^{bA}	<0.0001
9 weeks	31.11 ± 0.70^{aB}	16.22 ± 2.19^{bB}	9.05 ± 1.18^{cB}	<0.0001
p-value	<.0001			
Interaction	0.001			
Abnormal sperms (%)				
6 weeks	39.70 ± 2.89^{aA}	23.14 ± 0.87^{bA}	16.94 ± 0.65^{cA}	<0.0001
9 weeks	34.46 ± 2.80^{aB}	18.38 ± 1.06^{bB}	11.22 ± 0.77^{cB}	<0.0001
p-value	0.002			
Interaction	NS			
Sperms concentration ($X10^{9}$/ml)				
6 weeks	0.61 ± 0.05^{cB}	1.72 ± 0.07^{bB}	2.72 ± 0.07^{aB}	<0.0001
9 weeks	1.25 ± 0.08^{cA}	2.32 ± 0.07^{bA}	3.30 ± 0.07^{aA}	<0.0001
p-value	<.0001			
Interaction	NS			
Ovary index weight (9wks)	3.20 ± 0.06^{c}	4.42 ± 0.06^{b}	5.13 ± 0.05^{a}	<0.0001

The data were presented as mean ± standard error. Means bearing different superscript small letters within the same row are significantly different ($p < 0.05$) for the treatment effect and those in the same column with different capital letters are significantly different ($p < 0.05$) for the time effect.

concentration were higher at 9 weeks than 6 weeks of age, whereas the percentage of dead and abnormal spermatozoa was higher at 6 weeks than 9 weeks. There was a significant ($p < 0.0001$) interaction between time and treatment on the mass motility, individual motility, and concentration of spermatozoa.

The effect of nano-Se on plasma testosterone level at 6 weeks and 9 weeks of age in male Japanese quail is shown in Figure 1. Plasma testosterone level was the highest in the plasma of birds in Group 3 as compared to the other two groups during both periods (6 and 9 weeks of age). Group 1 birds had the lowest level of plasma testosterone. There was a significant ($p < 0.0001$) variation between 6 and 9 weeks of age at a higher level at 9 weeks.

Data on the deposition of Se in the testes at 6 and 9 weeks of age are shown in Figure 2, whereas deposition

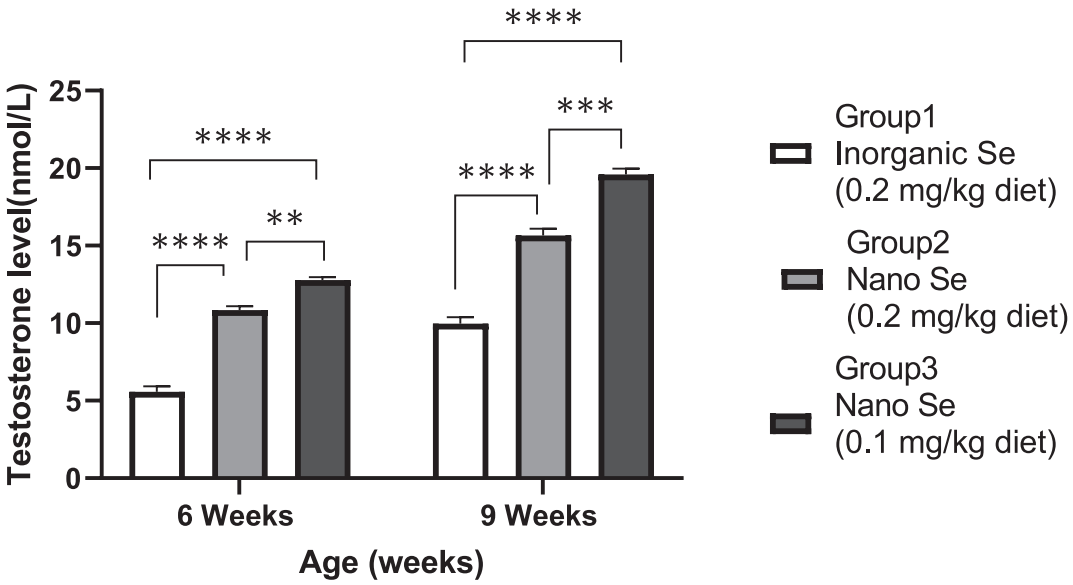

Figure 1. Effect of nano-selenium on plasma testosterone level in male Japanese quail at 6 and 9 weeks of age. ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

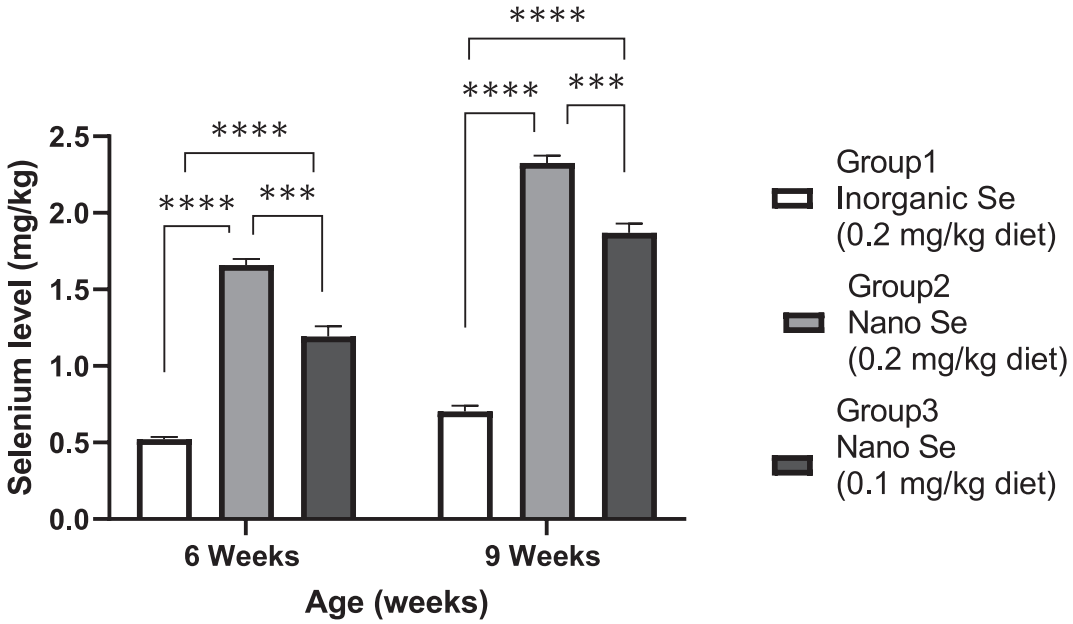

Figure 2. Deposition of selenium in the testes of male Japanese quail at 6 and 9 weeks of age. *** $p < 0.001$; **** $p < 0.0001$.

in the ovary is shown in Figure 3. Se deposition in testicular and ovarian tissues occurred in a concentration-dependent manner, where tissue deposition increased with increasing supplement concentration; however, even at the same concentration, nano-Se deposition was greater than that of inorganic Se.

Discussion

Various studies have shown that Se is the cofactor and activator of 5′-deiodinase that is a crucial enzyme of triiodothyronine (T3) synthesis. T3 has a role in the regulation of the growth in poultry by controlling the energy and protein assimilation in the body [14,35]. Another study reported that the use of Se in the diet (at 0.10–0.25 mg/kg) substantially increased the broiler body weight and decreased the feed efficiency ratio as compared to diet premixes that were prepared without Se and vitamin E [36].

In this study, nano-Se supplementation did not affect body weight and weight gain of Japanese quail. A similar observation in broilers showed that 0.3–2.0 mg/kg supplementation of nano-Se did not influence the growth performance [37]. Even at 8 mg/kg of dietary Se from 3 to 6 weeks of age, broiler weight and feed efficiency were not affected [38]. Another study suggested that nano-Se supplementation did not affect the body weight gain of broiler chickens [39–41]. However, in quails, 0.25-ppm nano-Se improved the final body weight, whereas higher inorganic Se negatively affected performance [42].

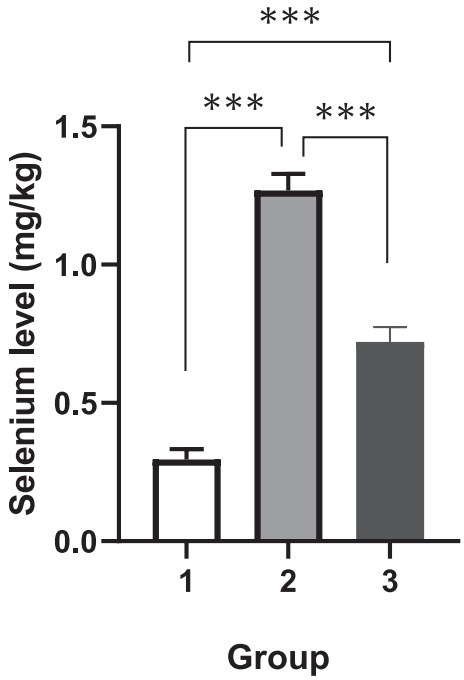

Figure 3. Effect of dietary selenium (Group 1: 0.2 mg/kg inorganic Se, Group 2: 0.2 mg/kg nano-Se, Group 3: 0.1 mg/kg Nano-Se) on Se level in the ovary of Japanese quail at nine weeks of age. *** $p < 0.001$.

In this study, we observed that 0.2 mg/kg nano-Se significantly reduced the total feed intake, FCR, PER, EEU, and PI, as reported by Divya et al. [43]. They found that 0.25-ppm nano-Se supplementation in Japanese quails had better feed efficiency than different levels of inorganic Se (0.2 and 0.5 ppm). Moreover, Boostani et al. [44] showed that nano-Se did not affect FCR. El-Deep et al. [41] reported that nano-Se non-significantly decreased FI and improved FCR.

The findings described that the productive performance was not affected by nano-Se, as supported by Radwan et al. [45], who found that egg weights were not affected by nano-Se. Furthermore, Payne et al. [46] found that average hen-day egg production was not influenced by the source of Se in the diet. Several studies [10,12,16] have also documented that egg production percentage was not affected by Se level. Other reports described that dietary nano-Se supplementation could improve the productive performance of layers and Turkey hens [47–49]. The triiodothyronine (T3) hormone regulates metabolism and growth [50]. In addition, Se is an essential part of GSH-Px, which decreases the free radicals from metabolism. The increase in free radicals is associated with low productivity [3].

We found that Se nanoparticles caused a marked increase in the expression of sexual and mating behavior in birds. The behavior was more marked in the lower concentration of nano-selenium (at 0.1 mg/kg diet). The improvements in sexual behavior could be attributed to the positive roles of Se in reproduction [13,51], particularly its enhancement of semen quality by increasing the activity of the antioxidant enzyme GSH-Px, and the synthesis of them requires Se [52]. The enzyme also involved supporting spermatogenesis and sperm functions [53] and testosterone biosynthesis [18].

Testosterone is the primary sex steroid in the avian testis and controls sexual maturity and sexual behavior. In this study, nano-Se significantly increased testosterone levels in the plasma as compared to the control group. This also correlates with the increased uptake of the nanoparticles by the tissues [32,53]. Moreover, we observed that the increase in testosterone level was more significant for nano-Se supplemented at 0.1 mg/kg of diet than a 0.2 mg/kg diet. This is associated with increased expression of sexual behavior patterns in Group 3 (at 0.1 mg/kg diet) compared to Group 2 (at 0.2 mg/kg diet). The changes in the secretion and plasma level of testosterone affect the testicular activity and sexual behavior expression [17].

Moreover, the addition of Se to the diet increases serum testosterone levels in male broilers [18]. It increases sexual activity by decreasing mating and ejaculation times and increased mating frequency [54]. The studies suggest that a higher concentration (at 0.2 mg/kg) of nano-Se may have undesirable effects.

Selenium is considered to be a crucial dietary trace element, which has an antioxidant activity for the protection of the cell from oxidative damage. It is also an essential part of various proteins with catalytic and structural functions [4,14]. The significance of sperm assessment in the poultry industry for routine monitoring of their reproductive performance has been well recognized [30]. In general, testosterone is needed to maintain the weights of reproductive organs and accessory glands [30,54]. The present study revealed that nano-Se increased male and female reproductive organ weights and improved testicular functions with enhanced plasma testosterone levels. These effects, which are likely due to the antioxidant activity of Se and protection of the testicular tissue from oxidative damage and stress, are expected to improve the overall gonadal function [37].

The enhancement in testis weight revealed in this study with Nano-Se treatments could be due to the improvement in the thickness and area of the germinal layer in seminiferous tubules or to increased testosterone levels [55]. Meanwhile, the improvement of mass and individual motility may be correlated with the increased concentration of spermatozoa associated with the increased gonadal size. Se is localized on the mid-piece of sperms, and its reduction, according to the severity, leads to decreased sperm motility, abnormal sperm morphology, and infertility [1]. The alleviation of oxidative stress by Se is expected to reduce the percentage of dead/immotile spermatozoa and increases the rate of motile spermatozoa [14]. Nano-Se had essential roles in improving seminal plasma and testicular tissue by lowering the levels of malondialdehyde. The latter is a potent oxidation byproduct associated with an increased percentage of dead and abnormal spermatozoa [55]. An increase in sperm concentration is also positively correlated with sperm motility [54]. Accordingly, it has been recorded that sperms are predominantly vulnerable to oxidative damage owing to the abundance of plasma membrane polyunsaturated fatty acids. Moreover, Se is the main constituent of GSH-Px and selenoproteins confirming sperm viability and supplying protection against reactive oxygen species [52].

Improvements in plasma testosterone levels and reproductive parameters were more prominent in the lower level of nano-Se (at 0.1 mg/kg diet) as relative to the higher concentration (at 0.2 mg/kg diet). Alavi et al. [56] reported a similar finding, in which the semen quality of broilers improved with a lower concentration of supplements of trace minerals than in the higher concentrations. The present study provides baseline data to conduct additional studies to establish more optimal Se supplementation concentrations and to understand why the lower concentration of Se is more beneficial than that of the higher concentration. The deposition of Se in the testicular and ovarian tissues is increased in a concentration-dependent manner. This might be due to the improved uptake of the nanoparticles by the tissues and their diffusion across the cell membrane [4,33,57]. It has been shown that nano-Se absorption from the intestinal lumen is more heightened than sodium selenite. Furthermore, the dietary administration of nano-Se caused a higher Se concentration in broiler tissues, as compared to sodium selenite [56].

Conclusion

Body weight, feed efficiency in addition to egg production, and egg weight are not affected by nano-Se administration; however, at 0.2 mg/kg diet, nano-Se significantly reduces the total feed intake throughout the experiment. Nano-Se supplementation increases sexual behavior at the lower concentration (0.1 mg/kg diet) than at the higher (at 0.2 mg/kg diet) concentration. Moreover, Se deposition in testicular and ovarian tissues followed a concentration-dependent manner. The nano-Se deposition is higher than that of inorganic Se of the same concentration. Furthermore, nano-Se improves plasma testosterone levels and, subsequently, the sperm quality traits. The improvement is more pronounced at the lower (at 0.1 mg/kg diet) than the higher (at 0.2 mg/kg diet) nano-Se concentration. There are also other subtle (numerical but not statistically significant) improvements in egg production parameters and FCR. Finally, we conclude that the lower concentration of nano-Se is more beneficial; the evaluation of even lower concentrations of nano-Se as a supplement is warranted.

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Conflicts of interest

The authors declare that they have no conflict of interests.

Authors' contributions

SEK, MIA, MHH, and GW conceived the study and the design of the experiment. SEK, MIA, MHH, and SAE carried

out the fieldwork. SEK carried out the measurements of the behavioral pattern. MIA carried out all growth and productive performance measurements and also carried out the statistical analysis and figures. MHH performed all physiological, reproductive parameters, and gonadal Se determination. SAE carried out the nutritional measurements and ration preparation. All authors were involved in writing, revising the manuscript, and permitted the final revision.

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