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Selenium nanoparticles are more efficient than sodium selenite in reducing the toxicity of aflatoxin B_1 in Japanese quail

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Abstract

Background: Dietary selenium (Se), as an antioxidant element, plays a protective role in aflatoxin B_1 (AFB_1) toxicosis in poultry.

Objectives: To compare the effects of sodium selenite (SS) and Se nanoparticles (SeNPs) against AFB_1-induced toxicity on growth performance, carcass traits, immune response, antioxidant status and serum lipid concentrations in Japanese broiler quails.

Methods: A total of 540 quails were divided into six treatments, each with six replicates and 15 birds per replicate at 24 days of age and reared for 21 days. Treatments included: (1) a basal diet without Se and AFB_1 (negative control; NC); (2) NC + 1.0 mg/kg AFB_1 (positive control; PC); (3) PC + 0.2 mg/kg Se as SS; (4) PC + 0.5 mg/kg Se as SS; (5) PC + 0.2 mg/kg Se as SeNPs; and (6) PC + 0.5 mg/kg Se as SeNPs.

Results: Treatment with PC diet decreased feed intake and body weight gain and increased feed conversion ratio than the NC diet. The PC diet also atrophied the lymphoid organs and depressed antibody responses against Newcastle disease and avian influenza viruses and sheep red blood cell. Moreover, quails treated with PC diet appeared to have lower serum glutathione peroxidase and thioredoxin reductase activities and disturbed serum lipids than those receiving the NC diet. Dietary Se attenuated these detrimental effects, but failed to completely eliminate them. Additionally, SeNPs performed better than SS in improving thioredoxin reductase activity and antibody titer against sheep red blood cell.

Conclusions: Diet supplementation with SeNPs to provide 0.5 mg/kg of Se is recommended to reduce the AFB_1 toxicosis in broiler quails.

KEYWORDS
aflatoxin B_1, antioxidant status, broiler quail, immune response, performance, selenium source

1 | INTRODUCTION

Aflatoxins are a group of mycotoxins mainly produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Balina et al., 2018; Sukcharoen et al., 2019). The types B_1, B_2, G_1 and G_2 are the most spread aflatoxins, and among them, aflatoxin B_1 (AFB_1) is the most toxic (Kumar et al., 2017). Aflatoxicosis induced by AFB_1 negatively affects all production characteristics of commercial birds in both males and females. The most presenting signs are growth retardation, anorexia, low egg production and hatchability, susceptibility to microbial and environmental stresses and an increase in mortality rate in Japanese quails (Nazar et al., 2012; Oliveira et al., 2002). The significant impact of aflatoxicosis on the carcass occurs first in the liver and then in the kidneys and lymphoid organs (Attia et al., 2016). This

leads to metabolic derangements including depression of protein, DNA and RNA synthesis (Chen et al., 2016), disorder of lipid metabolism (Ugbaja et al., 2020), loss of mitochondrial function, increased free radical production (Dwivedy et al., 2018) and immunosuppression (Mahmood et al., 2017). Accordingly, dietary supplementation with antioxidants may have the ability to protect poultry against AFB_1-induced toxicosis.

Selenium (Se) is an essential trace element with multiple functions that may help mitigate adverse health conditions. It is an integral part of at least 30 distinct selenoproteins, most of which have roles in detoxification and maintaining the redox potential (Habibian et al., 2015). Se also plays a role in lipid metabolism, and Se deficiency is associated with abnormalities related to plasma lipid and lipoprotein profiles (Xu et al., 2017). Besides, Se is essential for the efficient and effective operation of many aspects of the immune system in poultry (Attia et al., 2010; Dalgaard et al., 2018; Nabi et al., 2020; Shabani et al., 2019). Dietary Se supplementation acted against AFB_1 toxicity by increasing the feed intake and body weight gain (Jakhar et al., 2001; Ulaiwi, 2017), improving the ability to synthesize protein and DNA (Sun et al., 2016) and enhancing the immunocompetence of the body (Chen et al., 2013; Chen, Fang et al., 2014; Chen, Peng et al., 2014; Yu et al., 2015) in broiler chickens and quails. However, there are inconsistencies in the results from different studies. For example, Ulaiwi (2017) found that supplementation of up to 1.6 mg/kg of Se in the diet reduced the harmful impacts of 0.8 mg/kg of AFB_1 on the performance in Japanese quails, whereas Talebi et al. (2017) reported that 0.3 and 0.6 mg/kg of dietary Se equally helped to reduce the immunosuppressive effect of 1.0 mg/kg of AFB_1 in the quails of the same age. Otherwise, Chen et al. (2013) showed that even a level of 0.4 mg/kg of dietary Se supplementation could exacerbate the harmful effects of AFB_1 poisoning (0.3 mg/kg) on the immune and antioxidant functions in broiler chickens. Furthermore, preliminary evidence suggested that the use of Se in the form of nanoparticles (SeNPs) is better than sodium selenite (SS), the most commonly used form Se additive, in improving the immune status of broiler quails (Talebi et al., 2017). According to Nabi et al. (2020), SeNPs can enter the body more quickly and have better mobility inside the body than SS. Additionally, SeNPs appear to be less toxic than SS while possessing a higher or equal efficiency in the saturation of the Se stores and upregulation of selenoenzymes (Mohapatra et al., 2014; Pardechi et al., 2020). However, information on performance and antioxidant status in aflatoxin-exposed quails fed SeNPs is still limited.

Based on this background, the present study was carried out to compare the effects of SS and SeNPs against AFB_1-induced toxicity on growth performance, carcass traits, immune response, antioxidant status and serum lipid concentrations in Japanese broiler quails.

2 | MATERIALS AND METHODS

2.1 | Se sources and AFB_1 preparation

Anhydrous SS (99% purity) was from Sigma (Sigma-Aldrich, St. Louis, MO, USA) and was purchased from Damyar Jame Company (Tehran, Iran). The stable SeNPs were prepared following the green method of Ramamurthy et al. (2013). Briefly, 1 ml of fenugreek seed extract was mixed with 10 ml of 30 mM selenious acid solution, along with 200 μl of 40 mM ascorbic acid, which was used as an initiator of the reduction reaction. After 24 h of incubation at room temperature, the preparation was centrifuged at 8000 × *g* for 30 min. The pellet was washed with double-distilled water and then with absolute ethanol three times. The ethanol-washed pellet was dried overnight. The red SeNPs were suspended in phosphate buffer saline solution (pH 7.4) by ultrasonication and centrifuged. The powder form of the extract was used for further analysis. The size distribution of the SeNPs was analyzed by dynamic light scattering with a zeta potential analyzer (Malvern Instruments, Malvern, UK) and found to be in the range of 10–30 nm for 10%, 30 to 100 nm for 85% and 100 to 150 nm for 5% of the particles (Figure 1a). Transmission electron microscopy (Philips FEI, Oregon, OR, USA) confirmed these observations and showed that the particles are spherical (Figure 1b). Total Se concentrations were 449 mg/kg in SS and 386 mg/kg in SeNPs, respectively, as analyzed using an AAnalyst-100 atomic absorption spectrometry (Perkin Elmer, Norwalk, CT, USA) equipped with a deuterium background corrector and a graphite furnace (Model HGA-2100, Perkin Elmer). The analyses were carried out at 196 nm. A more detailed description of procedures can be found in Demirci and Pometto (1999).

The AFB_1 was produced by growing a standard strain of *Aspergillus flavus* (PTCC 5286) on sterile polished rice according to a modified method of Shotwell et al. (1966). Rice was cleaned, washed and autoclaved at 121°C for 15 min, dispensed in 500-ml Erlenmeyer flasks, and moistened with distilled water (10 ml/flask). Each flask was injected with 10 ml of fresh saline spore suspension containing 10^8 spores/ml. Flasks were incubated for 4 days at 18°C, followed by 3 days at 26°C. The flasks were vigorously shaken and sterilized by autoclaving to kill the fungus and its spores, while the toxins were preserved. The rice was dried and ground in an electric blender until powdered. The AFB_1 content was determined using the HPLC (Agilent 1100, Waldbronn, Germany) based on the AOAC method 991.31 (AOAC, 2000).

2.2 | Birds husbandry and dietary treatments

Five hundred and forty 20-day-old male Japanese quail chicks (97.8 ± 2.33 g) were obtained from a commercial supplier and housed in cages (36 cages: 0.15 m^2) in an environmentally controlled room. The cages were supplied with one water trough, two trough feeders, and wood shavings as bedding materials. The house temperature was set at 24°C and the relative humidity was 50% for the duration of the experimental period. Birds were exposed to a lighting regimen of 18 h light:6 h dark.

The chicks were divided into six groups, each with six replicates of 15 birds. The dietary treatments were as follows:

1. a basal diet without AFB1 and Se additives (negative control [NC])
2. NC + 1.0 mg/kg AFB1 (positive control [PC])
3. PC + 0.2 mg/kg Se in the form of SS

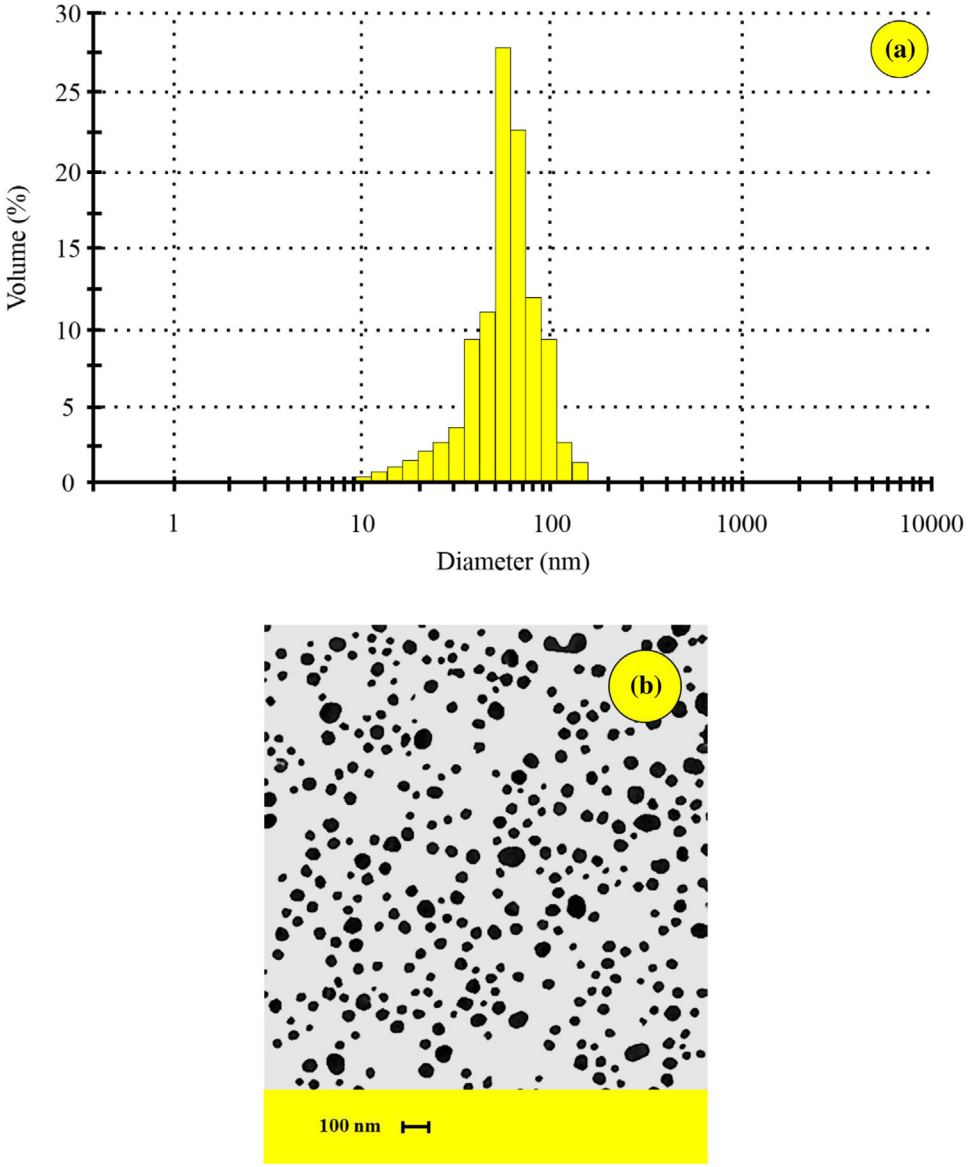

FIGURE 1 Size characterization of selenium nanoparticles by (a) dynamic light scattering and (b) transmission electron microscopy

4. PC + 0.5 mg/kg Se in the form of SS
5. PC + 0.2 mg/kg Se in the form of SeNPs
6. PC + 0.5 mg/kg Se in the form of SeNPs

The NC diet (Table 1) was formulated to meet NRC (1994) nutrient requirements. The contaminated rice powder was added at the expense of uncontaminated rice powder to the NC diet to provide the desired amount of AFB_1. The level of 1.0 mg/kg AFB_1 was chosen based on works by Mahmood et al. (2017) and Talebi et al. (2017), in which this dose caused significant adverse effects on quail performance and immune status. Supplemental Se levels were selected based on Surai et al. (2018). The experimental period started at 24 days of age and lasted until 45 days of age. From 20 to 24 days, all birds were fed the NC diet to adapt to their diet and environmental conditions. The birds were given unrestricted access to feed in mash form and water throughout the experiment.

2.3 | Sampling and measurements

Body weight and feed intake were recorded daily and mortality was recorded as it occurred. From these data, average daily feed intake, daily body weight gain, and feed conversion ratio were calculated per cage and analyzed every 7 day. Percent mortality was calculated for the entire period.

At the end of the experiment, 12 quails per treatment (two birds per replicate) were chosen, based on their body weight, and killed by exsanguination after electrical stunning to determine the relative weights (% of body weight) of the carcass, liver, heart, proventriculus, gizzard, small intestine (duodenum, jejunum, ileum), ceca, bursa of Fabricius, spleen and thymus. Also, the length of the small intestine and ceca was measured.

All quails received the intramuscular injection of a dual vaccine of Newcastle disease virus (NDV) and avian influenza virus (AIV) at 7 days

TABLE 1 Ingredient and nutrient contents of the basal diet (g/kg, or as noted)

Ingredients		Calculated composition		Analyzed composition[3]	
Corn	510.25	Metabolizable energy (MJ/kg)	12.13	Gross energy (MJ/kg)	16.84
Soybean meal	350.00	Crude protein	240.87	Dry matter	913.86
Corn gluten meal	75.30	Methionine	5.25	Crude protein	243.76
Polished rice[1]	22.10	Methionine + Cysteine	7.53	Ether extract	36.49
Soybean oil	6.40	Lysine	13.25	Neutral detergent fiber	110.27
Calcium carbonate	13.65	Threonine	10.12	Acid detergent fiber	44.74
Dicalcium phosphate	9.76	Tryptophan	2.20	Ash	57.41
Common salt	2.66	Calcium	8.06	Calcium	9.05
Sodium bicarbonate	1.37	Available phosphorus	3.09	Total phosphorus	5.49
L-Lysine-HCl	2.10	Sodium	1.50	Selenium (mg/kg)	0.24
L-threonine	1.41	Potassium	1.42	Aflatoxin B_1	ND[4]
Vitamin-mineral premix[2]	5.00	Chlorine	1.40		

[1]The contaminated rice powder was added at the expense of uncontaminated rice powder to the basal diet to provide the desired amount of AFB_1 in the experimental diets.
[2]The vitamin-mineral premix provided the following quantities per kg of diet: vitamin A, 1650 IU (all-trans-retinal); cholecalciferol, 750 IU; vitamin E (as dl-α-tocopheryl acetate), 10.8 mg; vitamin K_3, 1.0 mg, thiamin, 2.0 mg; riboflavin, 4.0 mg; niacin, 40 mg; D-calcium pantothenic acid, 35 mg; vitamin B_6, 3.0 mg; biotin, 0.3 mg; choline, 2000 mg; vitamin B_{12}, 0.003 mg; folic acid, 1.0 mg; manganese (as $MnSO_4 \cdot H_2O$), 60 mg; iron (as $FeSO_4 \cdot 7H_2O$), 120 mg; zinc (as ZnO), 25 mg; copper (as $CuSO_4$), 5 mg; iodine (as $Ca(IO_3)_2$), 0.3 mg.
[3]Dry matter (method 934.01), crude protein (method 954.01), ether extract (method 920.39), ash (method 942.05), calcium (method 968.08), phosphorus (method 965.17) and AFB_1 (method 991.31) were determined as per AOAC (2000) and gross energy was measured by an Adiabatic Bomb Calorimeter (Gallenkamp autobomb, Leicestershire, UK). Neutral detergent fibre and acid detergent fibre were determined according to the procedures of Van Soest et al. (1991), and sodium sulfite was used in the assay. Selenium was determined by atomic absorption spectrometry (Demirci & Pometto, 1999).
[4]ND, not detected.

and then the NDV vaccine orally (via drinking water) again at 14 days of the experiment. Also, two birds per replicate were given an intravenous injection of 1 ml of 0.5% sheep red blood cell (SRBC) solution at 7 and 14 days of the experiment. Blood was taken from birds 7 days after each injection. Samples were centrifuged at 1500 × *g* for 10 min and sera were stored at −20°C. Antibody titers against NDV, AIV and SRBC were quantified by microtitration methods previously used by other researchers (Wegmann & Smithies, 1966; King & Hopkins, 1983). Titers were expressed as $\log_2$ of the reciprocal of the last dilution in which agglutination was observed.

Serum was assayed for the activity of glutathione peroxidase (GPx) according to the method of Rice-Evans and Miller (1994) and for the activity of thioredoxin reductase (TrxR) using the technique of Luthman and Holmgren (1982), while the content of malondialdehyde (MDA) in serum was assessed based on the method of Buege and Aust (1978).

Serum concentrations of triglycerides, total cholesterol, low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol were determined using the Pars Azmun kits (Pars Azmun Company, Tehran, Iran) and a spectrophotometer (Shimadzu UV-1800, Tokyo, Japan) according to the instructions mentioned on the kits by the manufacturer.

2.4 | Statistical analysis

Collected data were subjected to analysis of variance (ANOVA) based on a completely randomized design using the GLM procedure of SAS 9.1. Control dietary treatments (NC and PC) were compared with Se dietary treatments following a one-way ANOVA: $X_{ij} = \mu + T_i + e_{ij}$, where μ = overall mean, T_i = effect of treatment, and e_{ij} = residual random error, whereas the effects of dietary Se sources and levels were analyzed following a two-way ANOVA: $X_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk}$, where μ = overall mean, A_i = effect of Se source, B_j = effect of Se level, $(AB)_{ij}$ = interaction between Se source and level, e_{ijk} = residual random error. Cage served as the experimental unit and mean differences were determined using Tukey's studentized range test ($p < 0.05$). Percentage mortality data were transformed to square root before analysis.

3 | RESULTS

3.1 | Performance traits and mortality

The effects of dietary treatments on average daily feed intake and weight gain of quails are depicted in Table 2, while the results on feed conversion ratio and mortality percentage are illustrated in Table 3. Feed intake and body weight gain decreased with the PC diet than the NC diet throughout the experimental period ($p < 0.05$). The addition of Se to the diet could increase feed intake. Still, the feed intake recorded with the Se-supplemented diets was intermediate between those obtained with the NC and PC diets and was not significantly different from either. The daily weight gains obtained with Se-supplemented diets were equal to those achieved with the NC diet during the first 7 days but began to decline after that ($p < 0.05$). However, they remained

TABLE 2 Effects of aflatoxin B_1 (AFB_1) and selenium (Se) supplementation on body weight and daily weight gain in broiler Japanese quails at different periods of the experiment (24–45 days of age).[1,2]

Dietary treatments				Daily feed intake (g)				Daily weight gain (g)			
	AFB_1 (mg/kg)	Se source	Se level (mg/kg)	day 1–7	day 7–14	day 14–21	day 1–21	day 1–7	day 7–14	day 14–21	day 1–21
NC	–	–	–	27.39^{a}	28.69^{a}	28.56^{a}	28.18^{a}	4.80^{a}	4.40^{a}	4.08^{a}	4.43^{a}
PC	1.0	–	–	25.59^{b}	25.78^{b}	25.83^{b}	25.74^{b}	4.06^{b}	3.56^{c}	2.60^{c}	3.41^{c}
	1.0	SS	0.2	26.53^{a,b}	26.35^{a,b}	26.38^{a,b}	26.42^{a,b}	4.62^{a}	3.55^{b}	3.47^{b}	3.88^{b}
	1.0	SS	0.5	26.53^{a,b}	27.06^{a,b}	26.52^{a,b}	26.70^{a,b}	4.56^{a}	3.74^{b}	3.50^{b}	3.93^{b}
	1.0	SeNPs	0.2	26.27^{a,b}	26.28^{a,b}	26.94^{a,b}	26.50^{a,b}	4.44^{a}	3.68^{b}	3.69^{b}	3.94^{b}
	1.0	SeNPs	0.5	26.97^{a,b}	26.88^{a,b}	26.32^{a,b}	26.72^{a,b}	4.53^{a}	3.73^{b}	3.62^{b}	3.96^{b}
Main effects of Se											
		SS		26.53	26.71	26.45	26.56	4.59	3.64	3.49	3.91
		SeNPs		26.62	26.58	26.63	26.61	4.49	3.71	3.66	3.95
			0.2	26.40	26.32	26.66	26.46	4.53	3.62	3.58	3.91
			0.5	26.75	26.97	26.42	26.71	4.55	3.73	3.56	3.95
Pooled SEM				0.217	0.216	0.209	0.178	0.065	0.075	0.100	0.057
p-value											
Dietary treatments				0.030	<0.001	<0.001	<0.001	0.018	0.004	<0.001	<0.001
Se source				0.858	0.759	0.681	0.883	0.478	0.694	0.385	0.580
Se level				0.488	0.130	0.584	0.455	0.917	0.471	0.927	0.626
Se source × Se level				0.486	0.895	0.386	0.930	0.605	0.663	0.797	0.859

[1]Data represent the mean of six replicate pens per treatment.
[2]NC, negative control; PC, positive control; SS, sodium selenite; SeNPs, selenium nanoparticles (SeNPs); SEM, standard error of the mean.
[a–c]Means within row with different superscripts are significantly different ($p < 0.05$); Tukey's studentized range test was applied to compare means.

significantly higher than those detected with the PC diet ($p < 0.05$). The effects of AFB_1 and Se on feed conversion ratio were similar to that of daily weight gain. The increasing impact of AFB_1 on mortality was eliminated by adding Se to the diet ($p < 0.05$). No differences between the Se sources and Se levels were recorded on mortality and performance traits.

3.2 | Carcass traits

As shown in Table 4, the relative weights of the carcass and heart did not respond to the addition of AFB_1 and two levels of Se in the form of SS or SeNPs. However, the PC diet increased the relative weights of the liver and gizzard compared with the NC diet ($p < 0.05$). Dietary Se addition decreased these effects of AFB_1, and a higher dose of 0.5 mg/kg was more effective than 0.2 mg/kg ($p < 0.05$). Moreover, the relative weight of the gizzard produced a more significant response to SeNPs than SS ($p < 0.05$). On the other hand, the PC diet significantly reduced the relative weights of the bursa, spleen and thymus ($p < 0.05$). Se supplement from both sources had a positive effect in increasing the weight of all three lymphoid organs and its impact on thymus weight increased with an increase in dosage ($p < 0.05$). In terms of the relative weight of the proventriculus and the relative weights and lengths of the intestines no extensive change was observed among the treatment groups (data not shown).

3.3 | Antibody responses

The effects of treatments on antibody responses against AIV, NDV and SRBC are shown in Table 5. Feeding the PC diet significantly reduced the antibody titers than the NC diet at 14 and 21 days of the experiment ($p < 0.05$). The inclusion of Se in the diet significantly attenuated this effect of AFB_1 ($p < 0.05$) and the impact of SeNPs on SRBC titers was more marked than SS ($p < 0.05$). Moreover, while no difference between the two levels of Se was noticed on the AIV titers, 0.5 mg/kg of Se worked more effectively than 0.2 mg/kg in increasing the NDV titers at 14 days and in improving the SRBC titers at 14 and 21 days of the experiment ($p < 0.05$).

3.4 | Serum antioxidant status and lipid concentrations

According to Table 6, the PC diet decreased serum GPx and TrxR activities and increased MDA contents compared with the NC diet at 21 days of the experiment ($p < 0.05$). Treatment with Se levels caused a gradual decrease in MDA contents and increased GPx and TrxR activities ($p < 0.05$), although it failed to restore the oxidative indices to the NC levels ($p < 0.05$). Furthermore, the effects of SeNPs on MDA content and TrxR activity were more remarkable than SS ($p < 0.05$).

TABLE 3 Effects of aflatoxin B_1 (AFB_1) and selenium (Se) supplementation on daily feed intake, feed conversion ratio, and mortality in broiler Japanese quails at different periods of the experiment (24–45 days of age).[1,2]

Dietary treatments				Feed conversion ratio (g)				Mortality (%)
	AFB_1 (mg/kg)	Se source	Se level (mg/kg)	d 1–7	d 7–14	d 14–21	d 1–21	d 1–21
NC	–	–	–	5.73[b]	6.53[c]	7.04[c]	6.37[c]	0.00[b]
PC	1.0	–	–	6.34[a]	7.31[a]	10.08[a]	7.56[a]	2.83[a]
	1.0	SS	0.2	5.75[b]	7.50[b]	7.79[b]	6.81[b]	0.83[b]
	1.0	SS	0.5	5.82[b]	7.34[b]	7.72[b]	6.80[b]	0.33[b]
	1.0	SeNPs	0.2	5.93[b]	7.17[b]	7.36[b]	6.74[b]	0.83[b]
	1.0	SeNPs	0.5	6.01[b]	7.26[b]	7.31[b]	6.75[b]	0.33[b]
Main effects of Se								
		SS		5.79	7.42	7.75	6.80	0.58
		SeNPs		5.97	7.21	7.34	6.75	0.58
			0.2	5.84	7.33	7.57	6.78	0.83
			0.5	5.92	7.30	7.52	6.77	0.33
Pooled SEM				0.087	0.124	0.236	0.072	0.208
p-value								
Dietary treatments				0.035	0.002	<0.001	<0.001	<0.001
Se source				0.323	0.530	0.351	0.583	0.999
Se level				0.689	0.910	0.898	0.981	0.213
Se source × Se level				0.985	0.703	0.991	0.882	0.999

[1]Data represent the mean of six replicate pens per treatment.
[2]NC, negative control; PC, positive control; SS, sodium selenite; SeNPs, selenium nanoparticles (SeNPs); SEM, standard error of the mean.
[a–c]Means within row with different superscripts are significantly different ($p < 0.05$); Tukey's studentized range test was applied to compare means.

The PC diet increased serum concentrations of triglycerides, total cholesterol, and LDL cholesterol and decreased serum HDL cholesterol concentration than the NC diet ($p < 0.05$). These detrimental effects of AFB_1 were markedly relieved by adding Se to the diet, but they could not be fully restored to the NC levels ($p < 0.05$). There were no significant main effects or interactions between Se source and Se level on serum lipid concentrations (Table 7).

4 | DISCUSSION

Over the course of the present study, the addition of AFB_1 to the diet reduced the average daily weight gain by about 23.0% in growing broiler quails. This decrease might be a cause of reduced feed intake (about 8.6%) to avoid aflatoxicosis, as also reported in other studies (Khaleghipour et al., 2020; Mahmood et al., 2017). However, the increase in feed conversion ratio (about 18.7%) indicates that AFB_1 can also affect the digestion and absorption of nutrients or the metabolic fate of nutrients in the postabsorptive condition. This argument agrees with the reports of Han et al. (2008) and Chen et al. (2016), who showed that AFB_1 reduced protein digestibility and the activity of trypsin and chymotrypsin enzymes in broiler chickens. Aflatoxin may also impair DNA replication, RNA transcription and amino acid transduction processes that result in a general inhibition of protein synthesis and suppression of growth (Naseem et al., 2018; Sun et al., 2016). The addition of Se from both SeNPs and SS remarkably, but not wholly, reduced the effects of AFB_1 on the production traits (about 3.3%, 15.2% and 10.4% improvement in feed intake, body weight gain and feed conversion ratio, respectively) and no difference was noticed between the two sources and their levels. These results confirm the researches in broiler chickens that found the positive effects of SS in increasing feed intake and body weight (Attia et al., 2010; Jakhar et al., 2001) and improving the protein-synthesizing ability of the body (Sun et al., 2016). Our results also agree with the findings of other researchers who observed no difference between the effects of SeNPs and SS on broiler chicken performance (Boostani et al., 2015; Wang, 2009), although there are also findings on better effects of SeNPs on growth and feed conversion efficiency in broiler chickens and quails (Divya et al., 2019; Pardechi et al., 2020). However, these results are not directly comparable due to differences in treatment patterns.

The relative weights of the carcass, heart and proventriculus and the relative weights and lengths of the small intestine and ceca showed no change among the treatment groups. However, the addition of AFB_1 to the diet increased the relative weights of the liver (54.7%) and gizzard (68.0%). These results are reminiscent of those published by Huff and Doerr (1981), who showed that AFB_1 exerts a cytotoxic effect on the upper gastrointestinal organs that manifests as the enlargement of the gizzard size. Similarly, Quezada et al. (2000) and Magnoli et al.

TABLE 4 Effects of aflatoxin B_1 (AFB_1) and selenium (Se) supplementation on carcass traits (g/100 g of live body weight) in broiler Japanese quails at 21 days of the experiment (45 days of age).[1,2]

Dietary treatments										
	AFB_1 (mg/kg)	Se source	Se level (mg/kg)	Carcass	Liver	Heart	Gizzard	Bursa	Spleen	Thymus
NC	–	–	–	88.0	1.48^{c}	0.84	1.53^{b}	0.18^{a}	0.10^{a}	0.23^{a}
PC	1.0	–	–	86.4	2.29^{a}	0.87	2.57^{a}	0.12^{c}	0.06^{c}	0.16^{c}
	1.0	SS	0.2	88.6	$1.98^{a,b}$	0.90	2.12^{a}	$0.13^{b,c}$	$0.07^{b,c}$	$0.18^{b,c}$
	1.0	SS	0.5	87.8	1.78^{b}	0.90	$1.87^{a,b}$	0.15^{b}	$0.09^{a,b}$	$0.21^{a,b}$
	1.0	SeNPs	0.2	87.0	1.89^{b}	0.87	$1.93^{a,b}$	$0.14^{b,c}$	$0.08^{a,b,c}$	$0.17^{b,c}$
	1.0	SeNPs	0.5	86.3	1.81^{b}	0.81	1.69^{b}	0.15^{b}	$0.09^{a,b}$	$0.21^{a,b}$
Main effects of Se										
		SS		88.2	1.88	0.90	2.00^{a}	0.14	0.08	0.19
		SeNPs		86.6	1.85	0.84	1.81^{b}	0.15	0.08	0.19
			0.2	87.8	1.94^{a}	0.88	2.03^{a}	0.14	0.07	0.17^{b}
			0.5	87.0	1.80^{b}	0.86	1.78^{b}	0.15	0.09	0.21^{a}
Pooled SEM				0.30	0.038	0.013	0.076	0.003	0.002	0.004
p-value										
Dietary treatments				0.303	<0.001	0.217	0.001	<0.001	<0.001	<0.001
Se source				0.105	0.627	0.072	0.028	0.221	0.327	0.575
Se level				0.391	0.028	0.374	0.016	0.130	0.102	<0.001
Se source × Se level				0.965	0.332	0.361	0.586	0.221	0.327	0.575

[1]Data represent the mean of six replicate pens per treatment.
[2]NC, negative control; PC, positive control; SS, sodium selenite; SeNPs, selenium nanoparticles (SeNPs); SEM, standard error of the mean.
[a–c]Means within row with different superscripts are significantly different ($p < 0.05$); Tukey's studentized range test was applied to compare means.

(2011) reported that AFB_1 affects the liver in the form of increased fat and relative weight and a reduction of the overall secretory capacity of the organ. Dietary Se supplementation decreased the effects of AFB_1 on the liver and gizzard weights and the dose of 0.5 mg/kg was more effective than 0.2 mg/kg (21.6% vs. 15.5% reduction for liver and 30.7% vs. 21.2% reduction for gizzard). Additionally, the weight of the gizzard showed a more significant decrease with SeNPs than with SS (29.57% vs. 22.4%). These findings are consistent with the general protective role of Se in the liver and gastrointestinal tract (Placha et al., 2014). Sun et al. (2016) showed that the protective effect of Se against AFB_1 is mediated by inhibiting cytochrome P450 isozymes and enhancing the production of antioxidant selenoenzymes in the liver. Research in broiler chickens also found that Se supplementation in organic, inorganic and nano forms reduced the weight and fat content of the liver compared with a Se-deficient diet (Attia et al., 2010; Pardechi et al., 2020). In another study, the percentage weights of carcass, heart, liver and gastrointestinal organs were not affected by SeNPs and SS in broiler quails (Khazraie & Ghazanfarpoor, 2015). These discrepancies may be related to the differences in Se contents of the basal diets used and the degree of stress imposed.

On the other hand, the addition of AFB_1 to the diet suppressed the relative weights of the lymphoid organs (40.0%, 34.4% and 33.3% weight loss for spleen, thymus and bursa, respectively). These results align with the findings of Solcan et al. (2014) and Attia et al. (2016), who showed that diet contamination with AFB_1 induced a marked and progressive thymocytes depletion via the apoptotic process and reduced the relative weights of the bursa, spleen and thymus. Se supplement from both sources had a positive effect on increasing the weight of lymphoid organs (37.5%, 20.3%, and 18.8% improvement in the spleen, thymus and bursa weights, respectively) and its effect on thymus weight increased with an increase in Se dosage from 0.2 to 0.5 mg/kg (9.4% vs. 31.3%). These results can be explained based on the findings of Chen et al. (2013), Chen, Fang et al. (2014) and Chen, Peng et al. (2014), who showed that Se reduces the level of apoptosis in the lymphatic tissues by boosting the production of antioxidant enzymes and increasing the ability of the body to fight excess levels of reactive oxygen radicals. In confirmation of these findings, Hussain et al. (2004) reported that the induction of Se deficiency in broiler chickens harmed thymus and spleen development, and Boostani et al. (2015) reported that the use of Se in nano, organic and inorganic forms increased the weight of the spleen. Another study showed that although both SeNPs and SS were successful in boosting the weight of lymphoid organs, the effect of SeNPs was more pronounced, probably due to their higher absorption capacity and faster transfer to the targeted tissues (Pardechi et al., 2020). Investigating the cause of this difference needs further investigation.

Lymphoid organs play a vital role in the development and maturation of immunity in birds. Therefore, the present results are not surprising that AFB_1 suppressed antibody responses against AIV, NDV and SRBC (about 24.9%, 30.8% and 46.4%, respectively). Aflatoxin

TABLE 5 Effects of aflatoxin B_1 (AFB_1) and selenium (Se) supplementation on antibody titers against avian influenza virus (AIV), Newcastle disease virus (NDV), and sheep red blood cell (SRBC) in broiler Japanese quails at 14 and 21 d of the experiment (38 and 45 days of age).[1,2]

Dietary treatments				AIV (log_2)		NDV (log_2)		SRBC (log_2)	
	AFB_1 (mg/kg)	Se source	Se level (mg/kg)	day 14	day 21	day 14	day 21	day 14	day 21
NC	—	—	—	5.98^{a}	5.69^{a}	4.94^{a}	5.71^{a}	8.26^{a}	9.45^{a}
PC	1.0	—	—	4.52^{b}	4.25^{b}	3.40^{c}	3.97^{c}	4.21^{c}	5.31^{c}
	1.0	SS	0.2	5.21^{a,b}	4.71^{a,b}	4.16^{b,c}	4.20^{b,c}	5.89^{b,c}	6.68^{b,c}
	1.0	SS	0.5	5.25^{a,b}	4.70^{a,b}	4.35^{b}	4.43^{b}	6.84^{b}	7.60^{b}
	1.0	SeNPs	0.2	5.17^{a,b}	4.76^{a,b}	4.18^{b,c}	4.29^{b,c}	6.68^{b}	7.11^{b}
	1.0	SeNPs	0.5	5.21^{a,b}	4.55^{a,b}	4.38^{b}	4.61^{b}	7.14^{a,b}	8.51^{a,b}
Main effects of Se									
		SS		5.23	4.71	**4.25**	4.32	6.37^{b}	7.14^{b}
		SeNPs		5.19	4.66	4.28	4.45	6.91^{a}	7.81^{a}
			0.2	5.19	4.74	4.17	4.24^{b}	6.29^{b}	6.89^{b}
			0.5	5.23	4.63	4.37	4.52^{a}	6.99^{a}	8.05^{a}
Pooled SEM				0.058	0.062	0.087	0.083	0.191	0.200
p-value									
Dietary treatment				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Se source				0.723	0.522	0.874	0.258	0.041	0.034
Se level				0.728	0.158	0.222	0.027	0.025	0.002
Se source × Se level				0.991	0.198	0.974	0.698	0.487	0.488

[1]Data represent the mean of six replicate pens per treatment.
[2]NC, negative control; PC, positive control; SS, sodium selenite; SeNPs, selenium nanoparticles (SeNPs); SEM, standard error of the mean.
[a–c]Means within row with different superscripts are significantly different ($p < 0.05$); Tukey's studentized range test was applied to compare means.

may also reduce antibody response by inducing a general reduction in protein synthesis (Naseem et al., 2018). Besides, Celik et al. (2000) suggested that aflatoxin degrades and removes antibodies from the blood by inducing an increase in lysozyme enzymes in the liver and skeletal muscle. Like the present findings, Yunus et al. (2011) reported that aflatoxin contamination of the diet was associated with decreased antibody production and weakened innate immune systems in broiler chickens. An earlier study by the same authors showed a high correlation between the prevalence of Newcastle disease and broiler feed contamination with aflatoxins (Yunus et al., 2009). Dietary Se inclusion ameliorated this effect of AFB_1 (about 12.7%, 18.0%, and 49.2% increase in AIV, NDV and SRBC titers, respectively). In this context, the reduction in mortality by Se might be due to improved immunity. Moreover, the impact of SeNPs on the increase of SRBC titers was more significant than SS (55.6% vs. 42.8%) and 0.5 mg/kg of Se was more effective than 0.2 mg/kg in increasing the NDV titers at 14 days (28.4% vs. 22.6%) and in improving the SRBC titers at 14 (66.0% vs. 49.3%) and 21 days (51.7% vs. 29.8%) of the experiment. In line with this research, Chen, Fang et al. (2014) and Chen, Peng et al. (2014) observed that 0.3 mg/kg of supplemental dietary Se (as SS) improved serum concentrations of IgA, IgM and IgG. Talebi et al. (2017) also showed that the use of 0.3 mg/kg of Se as SeNPs was more efficient in comparison with the same amount of organic Se in inhibiting the destructive effects of AFB_1 on antibody titers against NDV and SRBC. Pardechi et al. (2020) showed that enhancing the antibody response against SRBC requires higher Se than AIV and the use of more available sources such as SeNPs is more suitable to achieve this goal. However, in their study, Se supplementation did not affect the NDV titer. They suggested that the effect of Se on the antibody response could depend on the target antigen type. However, it should also be borne in mind that even in the case of a particular antigen, the antibody response can be affected by the level, method and schedule of vaccination, as well as the species, age, sex and immune status of the flock (Khorajiya et al., 2015).

The addition of AFB_1 to the diet significantly reduced the activity of GPx (25.8%) and TrxR (59.0%) enzymes and increased the content of MDA (36.8%) in the serum. This finding confirms the results of Karimi et al. (2020), who reported that a combination of AFB_1, AFB_2, AFG_1 and AFG_2 in the diet significantly reduced the activity of superoxide dismutase and GPx enzymes in the liver of broiler quails. Yang et al. (2012) also showed that corn naturally contaminated with AFB_1 and AFB_2 in broiler diets reduced the liver activity of superoxide dismutase and GPx and increased lipid peroxidation products in the serum and liver. Se supplementation of 0.2 and 0.5 mg/kg concentration caused a decrease in MDA contents (7.7% and 14.0%, respectively) and increased GPx (10.1% and 20.4%, respectively) and TrxR (45.6% and 83.0%, respectively) activities, while the effects of SeNPs on MDA content and TrxR activity were more marked in comparison with SS (14.3% vs. 7.4% reduction in MDA content and 76.8% vs. 51.7% increase in TrxR activity). Induction of GPx activity by supplementing a suitable amount of Se in the diets of broiler birds is an excellent

TABLE 6 Effects of aflatoxin B_1 (AFB_1) and selenium (Se) supplementation on serum Se concentration and antioxidative status in broiler Japanese quails at 21 days of the experiment (45 days of age).[1,2]

Dietary treatments						
	AFB_1 (mg/kg)	Se source	Se level (mg/kg)	GPx (unit/mL)	TrxR (unit/ml)	MDA (nmol/L)
NC	—	—	—	74.6[a]	9.88[a]	3.86[d]
PC	1.0	—	—	55.3[c]	4.05[d]	5.28[a]
	1.0	SS	0.2	60.3[b,c]	5.43[c,d]	5.00[a,b]
	1.0	SS	0.5	66.4[b]	6.86[c]	4.78[a,b,c]
	1.0	SeNPs	0.2	61.6[b,c]	6.36[c]	4.75[b,c]
	1.0	SeNPs	0.5	66.8[b]	7.96[b]	4.30[c]
Main effects of Se						
		SS		63.3	6.15[b]	4.89[a]
		SeNPs		64.2	7.16[a]	4.52[b]
			0.2	60.9[b]	5.90[b]	4.88[a]
			0.5	66.6[a]	7.41[a]	4.54[b]
Pooled SEM				1.14	0.244	0.073
p-value						
Dietary treatments				<0.001	<0.001	<0.001
Se source				0.667	<0.001	0.008
Se level				0.010	<0.001	0.014
Se source × Se level				0.839	0.756	0.379

[1]Data represent the mean of six replicate pens per treatment.
[2]NC, negative control; PC, positive control; SS, sodium selenite; SeNPs, selenium nanoparticles (SeNPs); GPx, glutathione peroxidase; TrxR, thioredoxin reductase; MDA, malondialdehyde; SEM, standard error of the mean.
[a–d]Means within row with different superscripts are significantly different ($p < 0.05$); Tukey's studentized range test was applied to compare means.

protective response against oxidative damage under stressful conditions (Altan et al., 2003). Also, in Japanese quails treated with AFB_1, dietary supplementation with 0.2 or 0.5 mg/kg of Se in the form of SS could effectively inhibit the oxidative damage by enhancing the activity of GPx, TrxR and catalase in the liver (Sun et al., 2016). In another study, it was reported that the addition of SeNPs supplement to the diet of broiler chickens significantly increased the activity of GPx and inhibited the production of oxygen free radicals (Cai et al., 2012). Comparing the effects of SeNPs and SS, Pardechi et al. (2020) concluded that Se in the form of SeNPs was more effective in increasing TrxR activity in Se-deficient broiler chickens, while both supplements had the same effect on GPx activity. TrxR is a Se-dependent enzyme that, in addition to having a regulatory role in thiol homeostasis and cellular signaling, plays a central role in Se metabolism, reducing Se compounds and thereby providing selenide to synthesize all selenoenzymes (Madeja et al., 2005). Research has shown that TrxR also provides the selenide needed for its own synthesis (Hashemy et al., 2006). Therefore, TrxR is sensitive not only to dietary Se levels but also to the bioavailability and metabolism of the Se source (Čobanová et al., 2017).

In the study of the effect of different treatments on serum lipids, it was found that the levels of triglycerides, total cholesterol, and LDL cholesterol increased with the addition of AFB_1 to the diet (45.0%, 29.7% and 48.33%, respectively), while the opposite was true for HDL cholesterol (51.3% reduction). Adding Se supplements to the diet largely alleviated these harmful effects of AFB_1 by a 14.1% decrease in triglycerides, 12.6% decrease in total cholesterol, 6.5% decrease in LDL cholesterol and 34.7% increase in HDL cholesterol, which might be mediated by changes in the antioxidant status of the birds. It is generally accepted that LDL oxidation is the primary mechanism contributing to lipid disorders such as hypertriglyceridemia and hypercholesterolemia (Menéndez-Carreño et al., 2008; Petyaev et al., 2019) and fatty acid distribution changes (Yamaoka et al., 2008). Se plays a fairly well-recognized role in preventing LDL oxidation as part of the enzyme phospholipid-hydroperoxide GPx that is transported in the bloodstream by HDL (Steinbrenner & Sies, 2009). Sunde and Hadley (2010) showed that this enzyme maintains its activity at the lowest Se than other members of the GPx family. This finding may be why in the present study, there were no differences between different levels and sources of Se on serum lipid levels. In addition, Se deficiency has been shown to increase the activity of HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis, leading to elevated total cholesterol and LDL cholesterol and decreased HDL cholesterol in Se-deficient mice (Qu et al., 2000). These findings are not consistent with the study results that the concentrations of cholesterol and triglycerides in the blood were not significantly affected by using 0.2 and 0.4 mg/kg of Se as SS or selenomethionine in the diet of Japanese quail (Senobar-Kalati et al., 2019). This is more likely due to differences in stress levels between the two studies. In broiler chickens, Habibian et al. (2014) reported that 0.5 mg/kg of dietary Se prevented an increase in serum concentrations of triglycerides and total

TABLE 7 Effects of aflatoxin B_1 (AFB_1) and selenium (Se) supplementation on selected serum lipid concentrations (mg/dl) in broiler Japanese quails at 21 days of the experiment (45 days of age).[1,2]

Dietary treatments							
	AFB_1 (mg/kg)	Se source	Se level (mg/kg)	Triglycerides	Total cholesterol	LDL cholesterol	HDL cholesterol
NC	–	–	–	204[c]	297[c]	204[c]	72.5[a]
PC	1.0	–	–	296[a]	386[a]	303[a]	35.3[c]
	1.0	SS	0.2	256[b]	333[b]	279[b]	46.5[b]
	1.0	SS	0.5	254[b]	342[b]	285[b]	46.6[b]
	1.0	SeNPs	0.2	252[b]	334[b]	270[b]	48.9[b]
	1.0	SeNPs	0.5	255[b]	338[b]	299[b]	48.3[b]
Main effects of Se							
		SS		255	338	282	46.5
		SeNPs		254	336	284	48.6
			0.2	254	333	274	47.7
			0.5	254	340	292	47.4
Pooled SEM				3.6	3.8	5.4	1.42
p-value							
Dietary treatments				<0.001	<0.001	<0.001	<0.001
Se source				0.810	0.774	0.820	0.115
Se level				0.972	0.212	0.108	0.829
Se source × Se level				0.672	0.671	0.294	0.800

[1]Data represent the mean of six replicate pens per treatment.
[2]NC, negative control; PC, positive control; SS, sodium selenite; SeNPs, selenium nanoparticles (SeNPs); LDL, low-density lipoprotein; HDL, high-density lipoprotein; SEM, standard error of the mean.
[a–c]Means within row with different superscripts are significantly different ($p < 0.05$); Tukey's studentized range test was applied to compare means.

cholesterol in response to heat stress but had no effect on serum lipid concentrations under normal conditions.

5 | CONCLUSIONS

The present study showed that SS and SeNPs could relieve AFB_1 (1.0 mg/kg of diet) damage in broiler quails. Both SS and SeNPs, especially at 0.5 mg/kg of diet, improved growth performance, immune responses and serum lipids through antioxidant enzymes induction. Moreover, SeNPs showed more favourable effects on enhancing TrxR activity, serum MDA content and antibody titers against SRBC. Based on these results, supplementing the diet with SeNPs to provide 0.5 mg/kg of Se is recommended to reduce the toxic effects of AFB_1 in broiler quails.

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AUTHOR CONTRIBUTIONS

Seyed Kaveh Khazraei conducted the experiment and analyzed data. Sayed Ali Tabeidian supervised the project and edited the final version of the manuscript and was also involved with data curation; formal analysis; methodology; writing-review & editing. Mahmood Habibian advised the project, assisted with data interpretation, and wrote the first draft of the manuscript and was also responsible for conceptualization and formal analysis.

CONFLICT OF INTEREST

We declare that we have no financial or other competing interests.

ETHICS STATEMENT

Ethical approval was obtained from the Ethics Committee of the Islamic Azad University, Isfahan (Khorasgan) Branch (No. 162276792).

DATA AVAILABILITY STATEMENT

The original data of the paper are available from the corresponding author upon reasonable request.

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