Effect of nanosilicon dioxide on growth performance, egg quality, liver histopathology and concentration of calcium, phosphorus and silicon in egg, liver and bone in laying quails

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Abstract This experiment was conducted to evaluate the effects of different levels of nanosilicon dioxide (nSiO₂) on performance, egg quality, liver histopathology and concentration of calcium (Ca), phosphorus and silicon (Si) in egg, liver and bone in laying quails. The experiment was administered using 60 laying quails at 16–26 weeks of age with five treatments [0 (control), 500, 1000, 2000 and 4000 mg nSiO₂ per kg of diet] and four replicates in a completely randomized design. During the experiment, the amount of feed intake was recorded weekly and performance parameters were measured. During the last 3 days of the experiment, all of the eggs in each replicate were collected and egg quality parameters were measured. At the end of 26 weeks of age, the birds were sacrificed and blood samples were collected. Liver samples from each treatment were fixed in 10% buffered formalin for histopathological assessment. The right thigh bone and a portion of liver were inserted in plastic bags and stored at – 20. The results showed that nSiO₂ supplementation significantly affected egg weight and egg mass ($P < 0.05$). Also, dietary nSiO₂ supplementation decreased the yolk weight and increased the shell weight ($P < 0.05$). Moreover, nSiO₂ increased bone ash content, Ca and Si concentration in the bone ($P < 0.05$). The liver enzymes in plasma and the liver tissue histopathology were not significantly affected ($P > 0.05$) by dietary treatments. In conclusion, the results indicated that dietary supplementa-

tion of nSiO₂ could improve bone density and performance without any adverse effect on the health of laying quails.

Keywords Nanosilicon dioxide · Performance · Tissues minerals · Laying quails

Introduction

Silicon (Si) is the second abundant element in the earth's crust. This element exists in all body tissues, but it shows highest concentration in bone, skin, hair, nails and arteries (Jugdaohsingh 2007), and it is one of the essential elements for bone formation (Carlisle 1981; Nielsen and Sandstead 1974). It has been shown that Si supplementation in animals and humans increases bone mineral density and improves bone strength (Price et al. 2013). The deficiency of Si causes growth retardation, atrophy of organs, bone formation disorders, inflammation of the mucosa and of the skin in mammalian species (Bodak et al. 1997).

During the last few years, nanotechnology has been revealed as a fundamental approach in animal nutrition and especially in mineral nutrition. Nanoparticle is an expression used for particles with at least one dimension of 100 nm or less and represents an intermediate supramolecular state of matter (Maynard and Kuempel 2005). It is well known that due to high surface area and nanoscale size, nanoparticles possess unique physical and chemical properties (Sun et al. 2011).

Several studies have been conducted to evaluate the effects of silicate minerals on the performance and health of the poultry (Safaeikatouli et al. 2012). Tauqir et al. (2001) and Santurio et al. (1999) reported that poultry performance was improved with the use of silicate minerals in broiler diets. In turkeys, body weight gain and feed

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conversion ratio (FCR) significantly improved with dietary Si dioxide supplementation (Tran et al. 2015). In a study conducted by Carlisle (1972) the average daily weight gain for roosters fed with 100 mg of sodium meta-silicate for 25 days was 2.57 and 3.85 g in control and treatment groups, respectively. It has been proposed that silicate minerals increase digestibility of diet and performance in broilers via gastrointestinal tract stimulation (Safaeikatlouli et al. 2012). The most important silicate minerals, which are used in poultry diets, are kaolin, bentonite and zeolite. Various studies have demonstrated that sodium zeolite-A improved eggshell quality and bone strength (poultry and horse), enhanced tibia ash and decreased the incidence and severity of tibial dyschondroplasia in broilers fed diets with low levels of calcium (Ca) (Rabon et al. 1995). It is proposed that the beneficial effects of sodium zeolite-A on bone strength and eggshell might be related to its crystal structure or amounts of its Si or aluminum which affect Ca metabolism (Rabon et al. 1995). Therefore, we hypothesized that using Si in the form of nanoparticle increased its positive effects on the eggshell quality and performance of laying quails. Moreover, concentrations of Ca, phosphorus (P) and Si minerals in egg, liver and bone of laying quails have been investigated.

Materials and methods

Birds, management and treatments

All procedures used in this study were approved by the guidelines of the Animal Ethics Committee of the University of Kurdistan. A total of hundred 16-week-old laying Japanese quails (*Coturnix coturnix Japonica*) were obtained from a local supplier. The obvious runts and birds in extreme weights were eliminated within the first 2 weeks which was considered as adaptation period. At 18 weeks of age, 60 clinically healthy birds were selected and randomly distributed between 20 cages (20 × 20 cm; three birds per unit), with almost same average body weight (263 ± 8.9 g) and egg production rate ($87.1 \pm 2.22\%$) throughout the cages.

The birds were fed either a control diet without nSiO₂ addition, or diets containing 500, 1000, 2000, and 4000 mg nSiO₂/kg of diet until termination of the experiment. Four replicates were randomly assigned to each of the five dietary treatments. Feed (Table 1) and water were offered ad libitum. Light was provided for 16 h daily and temperature was maintained at 20 ± 2 °C throughout the experiment.

Table 1 Ingredients and nutrients composition of the experimental diets (% or as noted)

Item (% or as noted)	Supplemented nanosilicon dioxide level (mg/kg)				
	0	500	1000	2000	4000
Ingredients					
Corn	53.17	53.07	52.96	52.75	52.33
Soybean meal (44% CP)	35.00	35.02	35.04	35.08	35.16
Soybean oil	5.43	5.43	5.43	5.43	5.43
Limestone	3.79	3.82	3.86	3.93	4.06
Nanosilicon dioxide	–	0.05	0.10	0.20	0.40
Dicalcium phosphate	1.63	1.63	1.63	1.63	1.63
Sodium chloride	0.34	0.34	0.34	0.34	0.34
Vitamin-mineral premix ^a	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.14	0.14	0.14	0.14	0.14
Calculated composition					
Metabolizable energy (kcal/kg)	2900	2900	2900	2900	2900
Crude protein	20.00	20.00	20.000	20.00	20.00
Calcium	2.50	2.50	2.50	2.50	2.50
Available phosphorous	0.40	0.40	0.40	0.40	0.40
Sodium	0.15	0.15	0.15	0.15	0.15
Methionine	0.45	0.45	0.45	0.45	0.45
Analyzed value					
Silicon (mg/kg)	42	220	455	918	1842

^aThe vitamin-mineral premix provided the following quantities per kg of diet: vitamin A, 9000 IU; vitamin D₃, 2000 IU; vitamin E, 18 IU; vitamin K₃, 2 mg; vitamin B₁, 1.8 mg; vitamin B₂, 6.6 mg; vitamin B₃, 10 mg; vitamin B₅, 30 mg; vitamin B₆, 3 mg; vitamin B₉, 1 mg; vitamin B₁₂, 0.015 mg; biotin, 0.1 mg; choline chloride, 250 mg; antioxidant, 100 mg; Mn, 100 mg; Zn, 84.7 mg; Fe, 50 mg; Cu, 10 mg; I, 1 mg; Se, 0.2 mg

Sampling procedure

Production performance was measured from 18 to 26 weeks of age. Egg production and egg weight were recorded daily for each replicate, and the cumulative average egg production and egg weight calculated weekly. The collected data (number of eggs and egg weight) were used to calculate egg mass per replicate (egg number in replicate $\times$ average egg weight). Feed intake was measured on a weekly basis. Data on feed intake and egg mass were used to calculate feed conversion ratio (FCR, feed intake/egg mass, g/g). All the eggs laid during the last 3 days of the experiment, were collected by replicate (24 eggs per treatment) and yolk weight, albumen weight, shell weight, pH of yolk and albumen were measured. Furthermore, egg yolk relative weights (EYRW), albumen relative weight (AlbRW) and eggshell relative weight (shellRW) were calculated. To measure Ca, P and Si concentration of eggs, their contents (yolk and albumen) were mixed and stored at -20°C until further analysis. At the end of 26 weeks of age, access to feed was denied for a period of 10 h and blood samples were taken and the birds were sacrificed. The blood samples were transferred to heparinized tubes and centrifuged for 10 min at 3000 g. The plasma was removed and refrigerated (-20°C) until further analysis. After blood sampling, the birds were sacrificed, a portion of liver was obtained, fixed in 10% formalin, and transferred to the laboratory for histopathological study. Moreover, a portion of liver and right thigh bone inserted in plastic bags and stored at -20 until analysis for Ca, P, and Si contents.

Laboratory analysis

The activities of AST, LDH, and ALP in plasma were determined using a Jasco V-570 spectrophotometer (Jasco, Japan) as per the instructions of the corresponding reagent kit (Pars Azmun, Tehran, Iran). A representative sample of liver and egg contents from each pen was oven-dried at 70°C for 24 h, ground, and analyzed for mineral contents. The content of Si was determined by flame atomic absorption spectrophotometer (Phoenix-986, AA spectrophotometer, UK) after wet washing with concentrated hydrochloric acid (Pignalosa et al. 2001). The content of Ca was determined by the versenate complexometric titration method using ethylene diamine tetra acetic acid as indicator (AOAC 2000). The content of P was determined by the vanadium-molybdate method described by AOAC (2000). After removing any adhering tissue, thigh bones were fat extracted, and then analyzed for ash minerals (AOAC 2000) on a fat free dry basis. Bone Ca, P, and Si were determined as indicated in the mineral analysis of liver and egg contents. The liver samples were processed

for histological examination according to Bancroft and Gamble M (2008). The tissues were processed for paraffin embedding, and sections ($3\text{--}4\text{ }\mu\text{m}$) were stained with hematoxylin and eosin, and damaged tissue was evaluated using a light microscope.

Statistical analysis

The general linear model procedure of SAS software (SAS 2001) was used for analyzing the data in a completely randomized design model. The means of treatments were compared using Duncan's multiple range tests. Values of $P < 0.05$ were considered statistically significant.

Results

The effect of different levels of nSiO_2 on growth performance of laying quails during the 8-week study is shown in Table 2. Egg mass and egg weight of quails were increased by nSiO_2 (4000 mg/kg of diet) supplementation and were reduced significantly under level of 500 mg/kg nSiO_2 ($P < 0.05$). However, the feed intake, egg production and FCR of the laying quails were not affected by nSiO_2 supplementation ($P > 0.05$).

No significant difference ($P > 0.05$) was observed for the albumen weight, egg yolk relative weight (EYRW), albumen relative weight (AlbRW) and eggshell relative weight (shellRW), pH of yolk and albumen of quails after 8 weeks feeding with nSiO_2 supplementation (Table 3). However, there was a significant increase in the shell weight of quails fed diets containing 4000 mg nSiO_2 per kg of diet ($P < 0.05$). Moreover, yolk weight significantly decreased in the birds fed diets supplemented with 500 mg nSiO_2 per kg of diet ($P < 0.05$).

The effect of different level of nSiO_2 on bone, liver and egg minerals concentrations of laying quail is shown in Table 4. Supplemental nSiO_2 increased bone ash and Ca concentration in the bone of quails fed diets containing 4000 mg nSiO_2 per kg of diet ($P < 0.05$) and Si concentration of bone in quails fed diets containing 500, 1000 and 2000 mg nSiO_2 per kg of diet ($P < 0.05$). However, no significant effect was observed on Si, Ca, and P concentration in egg and liver samples ($P > 0.05$).

The effect of different level of nSiO_2 on concentrations (U/L) of liver enzymes in plasma in laying quails is shown in Table 5. No significant effect of treatments was found on any liver enzymes in plasma ($P > 0.05$). No significant change in the liver tissue histopathology was found among the treatments (Fig. 1).

Table 2 The effect of different levels of nanosilicon dioxide on performance of laying quails

Items	Supplemented nanosilicon dioxide level (mg/kg)					SEM	P values
	0	500	1000	2000	4000		
Feed intake (g/bird/day)	33.13	32.28	34.19	33.66	34.40	0.84	0.42
Egg production (%)	89.28	84.73	89.87	90.90	90.96	1.65	0.06
Egg weight (g)	12.72 ^{bc}	12.32 ^d	12.78 ^c	12.85 ^b	13.27 ^a	0.09	0.0001
Egg mass (g/bird/day)	11.35 ^b	10.47 ^c	11.37 ^b	11.83 ^{ab}	12.01 ^a	0/21	0.0001
Feed conversion ratio (g feed/g egg)	3.01	3.34	3.02	3.11	3.07	0.11	0.24

^{a-d}Means with same superscript in each row are not significantly different

SEM standard error of the means

Table 3 The effect of different levels of nanosilicon dioxide on egg quality of laying quails

Items	Supplemented dietary nanosilicon dioxide (mg/kg)					SEM	P values
	0	500	1000	2000	4000		
Yolk weight (g)	3.78 ^{ab}	3.60 ^c	3.72 ^{bc}	3.90 ^{ab}	4.08 ^a	0.07	0.001
Albumen weight (g)	7.96	7.64	8.00	7.79	8.23	0.15	0.11
Shell weight (g)	1.03 ^b	1.00 ^b	1.02 ^b	1.02 ^b	1.10 ^a	0.01	0.002
pH of yolk	5.95	5.86	5.88	5.91	5.87	0.02	0.07
pH of albumen	9.07	9.05	9.05	9.12	9.02	0.04	0.78

^{a,b}Means with same superscript in each row are not significantly different

SEM standard error of the means

Table 4 The effect of different levels of nanosilicon dioxide on ash, calcium, phosphorus and silicon contents in bone, liver and egg of laying quails

Items	Supplemented dietary nanosilicon dioxide (mg/kg)					SEM	P values
	0	500	1000	2000	4000		
Bone							
Ash (%)	56.53 ^{b*}	58.04 ^b	57.84 ^b	59.01 ^b	62.98 ^a	0.47	0.04
Calcium (%)	19.21 ^{bc}	19.43 ^{bc}	18.85 ^c	20.43 ^{bc}	21.77 ^a	0.23	0.02
Phosphorus (%)	9.09	9.24	9.17	9.27	9.13	0.10	0.94
Silicon (ppm)	1.87 ^c	11.78 ^a	10.51 ^a	7.94 ^{ab}	3.60 ^{bc}	1.41	0.01
Liver							
Calcium (%)	0.011	0.010	0.010	0.011	0.011	0.001	0.26
Phosphorus (%)	0.13	0.13	0.14	0.13	0.12	0.004	0.42
Silicon (ppm)	15.89	16.52	15.93	13.95	10.21	2.15	0.84
Egg							
Calcium (%)	0.015	0.015	0.013	0.013	0.015	0.003	0.18
Phosphorus (%)	0.79	0.76	0.78	0.73	0.81	0.02	0.88
Silicon (ppm)	1.53	1.76	3.35	2.84	2.70	0.43	0.70

*^{a,b}Means with same superscript in each row are not significantly different

SEM standard error of the means

Table 5 The effect of different levels of nanosilicon dioxide on concentrations (U/L) of liver enzymes in plasma of laying quails

Items	Supplemented dietary nanosilicon dioxide (mg/kg)					SEM	P values
	0	500	1000	2000	4000		
ALP	186.40	179.40	200.40	189.10	187.50	75.53	0.99
ASAT	166.56	192.20	181.72	195.37	204.06	19.20	0.30
LDH	317.45	278.65	207.97	349.19	283.46	68.42	0.48

ALP alkaline phosphatase, AST aspartate aminotransferase, LDH lactate dehydrogenase, SEM standard error of the means

Discussion

The results of the present study indicated that nSiO₂ supplementation increased quails egg weight and egg mass. Based on our knowledge, there is no published report related to use of nSiO₂ in laying birds. However, Tran et al. (2015) reported that in turkeys, supplementing diet with 0.02% SiO₂ significantly improved body weight gain and FCR. Moreover, it is reported that 100 mg/kg SiO₂ in the diet can improve growth rate in chickens (Carlisle 1972, 1976). An improvement in body weight gain could also be obtained with supplementation of 2500 mg/kg SiO₂ to diet of fattening chickens (Vanschoubroek and Vermeersch 1996). Although in the present study we did not measure nSiO₂ agglomeration, however, it is reported that high levels of nanoparticles in the diet, increase the possibility of their agglomeration in the small intestine leading to reduced absorption of the nanoparticles into the body (Philbrook et al. 2011; Bergin and Witzmann 2013). Sakai-Kato et al. (2014) reported that following a reduction in the electrostatic repulsion between negatively charged silica particles, agglomeration can occur in the stomach due to its lower pH conditions and as a result of agglomeration, intestinal absorption of the particles reduced. Chen et al. (2006) reported that nanoparticles increased the retention time of feed particles in the gastrointestinal tract and decreased intestinal emptying rate, and in turn improve nutrients absorption by intestinal absorptive cells. Moreover, Safaeikatouli et al. (2012), showed that silicate minerals make a temporary link with nutrient, decreases gastrointestinal transmission rate and therefore subject nutrients to further digestion which finally increases their absorption. As mentioned above, it is strongly possible that nSiO₂ agglomeration occurred in the small intestine of laying quails fed diets containing 4000 mg/kg which reduced their absorption and hence leading link with digesta nutrients and finally resulted in a significant reduction of nSiO₂ absorption in the small intestine. However in the birds treated with diets containing 1000 and 2000 mg/kg nSiO₂ compared to the control group, it seems that agglomeration was not enough to decrease

intestinal transmission rate and increase nutrients absorptions.

On the other hand, there was a significant reduction in the egg weight of the quails fed diets supplemented with 500 mg/kg nSiO₂, which can be due to negative effects of nSiO₂ on very low density lipoprotein (VLDL) as one of the major yolk-forming components. This hypothesis is strengthened by Peluso and Schheeman (1994), who reported that plasma very low density protein (VLDL), and low density lipoprotein (LDL) concentrations reduced with inclusion of silicates in the diet of rats. One of the most important compounds for VLDL production in the liver is cholesterol. Moreover, cholesterol is a substrate for the synthesis of bile acids (Bravo and Cantafora 1990) and previous studies showed that interaction between Si and bile acids in the intestine likely affects liver cholesterol homeostasis. Peluso and Schheeman (1994) showed that Si dioxide could increase fecal cholic acids excretion, which finally inhibits cholesterol biosynthesis through inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Therefore, reduction of vitellogenesis due to reduced VLDL biosynthesis (specific to production of egg-yolk) could be a reason for the reduced yolk weight and egg weights in birds treated with 500 mg/kg nSiO₂.

Although there is no previous study reported the effect of Si on eggshell formation in birds. However, it is demonstrated that Si has a significant effect on the bone matrix formation and bone calcification (Carlisle 1986, 1988). In our present study, there was a significant improvement in the eggshell weight of laying quails fed diets supplemented with 4000 mg/kg nSiO₂. Due to important role of stored excess calcium in the bone for eggshell formation in laying birds, it is likely that dietary nSiO₂ improved eggshell weight of laying quails via improving bone calcium reserve. Furthermore, it is likely that due to temporary link of Si with dietary calcium (Safaeikatouli et al. 2012), the amount of available calcium in the digestive tract during the period of eggshell formation increased and hence, improved eggshell thickness in the quails fed diets containing 4000 mg nSiO₂/kg (Farmer et al. 1986).

Fig. 1 Photomicrographs (optical microscopy) of hematoxylin and eosin-stained laying quail's liver sections from different treatments. **a** control; **b** contains 500 mg/kg nanosilicon dioxide; **c** contains 1000 mg/kg nanosilicon dioxide; **D** contains 2000 mg/kg nanosilicon dioxide; **E** contains 4000 mg/kg nanosilicon dioxide

In the present study, an increase in bone ash content and Ca concentration of the bone was observed in laying quails fed 4000 mg/kg nSiO_2 . As previously mentioned, agglomeration of Si nanoparticles at 4000 mg/kg of dietary nSiO_2 leading to an increase in nutrients absorption (e.g. Ca) in the small intestine. It is reported that Si particles are indestructible and burying them into the collagen tissue of the skeleton is the final solution (Pernis 2004). Hence, this could be an explanation for the increased concentration of

Si in the bone. Carlisle (1976) showed that percent of bone ash was not affected by Si supplemented diet. Our results were in concordance with Elliot and Edwards (1991) who reported that dietary Si supplementation has no effect on improvement and development of skeleton in broiler chickens.

Plasma biochemical parameters are the main tools for assessment of health in many species and are used as markers of disease or disorders in organs such as liver,

kidneys, and biliary tract (Tran et al. 2015). In the present study, no effects of treatments were observed on plasma parameters. Investigating the liver histopathology, hematoxylin and eosin staining showed no significant changes between treatments. In whole treatments, normal liver cells with red cytoplasm indicating active cells surrounded the central vein of the liver lobule showed the lack of damage in liver tissue in treated birds. Lack of histological lesions in the present study indicated the very low absorption of nSiO₂. Therefore, it is strongly possible that agglomeration of Si nanoparticles in the small intestine reduced its transfer to the liver. This assumption is supported by no significant effects of nSiO₂ on Si concentration in liver of birds.

Conclusions

In conclusion, supplementation of the laying quail's diet with nSiO₂ could improve bone density and performance of laying quails. In conclusion, supplementation the laying quail's diet with nSiO₂ could improve performance, egg shell weight and bone density of laying quails. According to the present study, it seems that supplementation of laying quail's diet with 4000 mg/kg nSiO₂ resulted in an optimal supply of necessary nutrients for egg weight via stimulation of the gastrointestinal tract; therefore the egg weight was increased.

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