The effects of dietary Se on productive and reproductive performance, tibial quality, and antioxidant capacity in laying duck breeders

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ABSTRACT This study evaluated the optimal concentrations of dietary Se for the productive and reproductive performance, tibial quality, and antioxidant status in duck breeders aged 23 to 49 wk. In total, 432 Longyan duck breeders aged 22 wk were allotted randomly to 6 treatments, each with 6 replicates of 12 individually caged birds. The experiment lasted for 27 wk, and birds were fed corn-soybean meal-based diets containing 0.11, 0.19, 0.27, 0.35, 0.43, or 0.51 mg Se/kg, respectively. The tested dietary Se levels did not affect egg production and tibial quality of duck breeders. The Se contents of the shell, yolk or albumin, whole egg, and the fertility of set eggs increased in a linear and quadratic manner ($P < 0.05$) in response to the increased dietary Se level, whereas the yolk malondialdehyde (**MDA**) and embryonic mortality decreased. The activities of glutathione peroxidase 3 (**Gpx3**) in plasma and Gpx1 in the erythrocytes and livers of breeder ducks increased in a linear and quadratic manner ($P < 0.05$) in response to increased dietary Se levels, whereas the total superoxide dismutase (**T-SOD**) activity increased and the MDA concentration decreased in the liver. The activity of Gpx3 in the plasma and Gpx1 in the erythrocytes and livers of newly hatched ducklings increased linearly ($P < 0.01$) with the increase in Se level, whereas the T-SOD activity and MDA concentration did not change. In conclusion, diets containing 0.27 mg Se/kg led to the highest egg fertility and hatchability in Longyan duck breeders, and using levels >0.19 mg Se/kg diet enhanced the antioxidant capacity in breeders and their offspring. The regression model indicated that dietary Se levels 0.19, 0.27, 0.28, 0.24, and 0.30 mg/kg are optimal levels to obtain maximum Se deposition efficiency in eggs, egg fertility, Gpx1 activity in erythrocytes and liver in duck breeders, and plasma activity of Gpx3 in newly hatched ducklings, respectively.

Key words: Se, duck breeder, reproductive performance, antioxidant capacity

2020 Poultry Science 99:3971–3978
https://doi.org/10.1016/j.psj.2020.04.006

INTRODUCTION

Poultry diets, generally, should contain balanced levels of around 38 nutrients (NRC, 1994). Using unbalanced diets or diets deficient in one or some nutrients reduces the anticipated performance of birds, and using an excess of nutrients, that is higher than the requirement, reduces production profits, and both defects may exert negative effects on bird's health. The main objective of this study was to estimate the optimal dietary Se level for laying duck breeders . Reviewing the recommendations of the NRC (1994), Se requirements are listed for broilers, laying hens, quail, and meat ducks (0–2 wk of age), but no estimates are available on Se requirements for laying ducks or duck breeders in NRC (1994). In laying ducks, Chen et al. (2015) found that the Se requirement for optimal daily egg production was 0.18 mg/kg feed during the early-laying period and 0.24 mg/kg during the peak-laying period. These

Received December 10, 2019.
Accepted April 1, 2020.

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available data on Se requirement for laying ducks are not adequate for duck breeders; duck breeders have different nutritional needs, where they have greater genetic capacity for egg production, quality, egg mass, fertility, and hatchability. It is necessary, therefore, to determine the optimal Se level for laying breeders of ducks to maximize their productive and reproductive performance.

A number of biological activities are dependent on the dietary Se level, such as cell growth, protein metabolism, and organ development (Brandt-Kjelsen et al., 2017). In the past, Se was foreseen as a toxic mineral for humans and animals (Gissel-Nielsen et al., 1984); however, its deficiency in animal feed leads to several negative effects including nutritional disorders, reduced growth, impaired egg production, poor feathering, low hatchability, increased pancreatic degeneration, and necrotic lesions in body organs such as liver and kidneys, resulting in large economic losses (Surai et al., 2018; Sun et al., 2019). Low dietary Se impairs the immune status of birds, which become more vulnerable to a list of diseases (Yang et al., 2016; Taha-Abdelaziz et al., 2018). Se is involved in the antioxidant property of the cell, regulation of thyroid secretions, immune function, and reproduction (Khan, 2011; Huang et al., 2016; Dalgaard et al., 2018). It is a vital component of selenoproteins, which include several glutathione peroxidases (**Gpx**) such as cellular Gpx (Gpx1) and phospholipid hydroperoxide Gpx (Gpx4), iodothyronine 50-deiodinases, sperm capsule selenoprotein, and thioredoxin reductase (Pappas et al., 2008). For accurate estimation of Se requirement, its functional properties are often considered, including the functional activity of selenoproteins as well as maximizing production performance.

The Longyan duck is a popular laying breed in China; originally, it is produced from crossing between the Longyan breed, Putian Black breed, and Putian White breed (Lin et al., 2014). In southern China, more than 300 million birds are raised from such breed, producing around 80 billion eggs per year, with an average adult BW of 1.5 kg and 280 to 300 eggs per duck yearly (Xia et al., 2015). Over the past 10 y, our group has been estimating nutrient requirements for the layers and breeders of Longyan duck breed, including CP, ME, amino acids, vitamins, and minerals (Chen et al., 2015; Xia et al., 2015; Xia et al., 2019a,b). As part of a systematic program for optimizing the performance of the highly productive laying duck breeders, this study aimed to estimate the optimal level of dietary Se, testing 6 graded levels, for maximal egg production, fertility, hatchability, tibial quality, and antioxidative status in Longyan duck breeders.

MATERIALS AND METHODS

Animals and Treatments

All procedures employed in this study were approved by the Animal Care and Use Committee of Guangdong Academy of Agricultural Sciences, Guangzhou, China (GAASIAS/No.2018-1029). A total of 432 Longyan duck breeders aged 22 wk with the same genetic background and comparable BW (1.40 ± 0.01 kg) were allotted randomly to 6 treatments, each with 6 replicates of 12 birds. Data collection started from 23 wk of age, and continued over the following 27 wk. The experimental ducks were housed in individual galvanized battery cages (length 40 cm × width 27.8 cm × height 55 cm, purchased from Guangzhou Huanan Poultry Equipment Co., Ltd., Guangzhou, China). Each battery cage was equipped with a feeder and nipple drinker. The ducks were fed corn-soybean meal basal diet (0.11 mg Se/kg, Table 1), supplemented with either 0, 0.08, 0.16, 0.24, 0.32, or 0.40 mg/kg Se in the form of sodium selenite (99.0% sodium selenite; Guangdong Newland Feed Science & Technology Co., Ltd., Guangdong, China). The final Se concentration in the tested diets, therefore, was 0.11, 0.19, 0.27, 0.35, 0.43, and 0.51 mg/kg, respectively. The other dietary nutrient levels in the basal diet were selected according to our previous findings for laying ducks (Xia et al., 2019b). Chemical and calculated analysis of the basal diet is shown in Table 1. Water was available ad libitum and 85 g of feed were introduced twice daily at 7:00 am and 3:00 pm throughout the experimental period (in total 170 g/bird/D). In addition to ambient daylight, 4 h of artificial light (15 lx/m^2) was provided from 6:30 pm to 10:30 pm to give in total 16 h light: 8 h dark a day.

Tissue Sampling and Storage

At the end of the study, 2 birds were randomly selected from each replicate and blood samples were collected from the left wing vein in 5 mL vacutainer tubes at 10:00 am after an overnight fast for 12 h. Within 30 min of collection, the blood was centrifuged (1,200 × *g* for 10 min) to separate plasma; the collected plasma was stored in 0.5 mL Eppendorf tubes at −20°C until analysis. Erythrocytes were ruptured by mixing 3 volumes of a Tris-HCl (1 mmol/L, pH 8.0) buffer solution with one volume of blood, agitated mildly in an ice bath, centrifuged (12,000 × *g* for 5 min) at 5°C to separate the supernatant cytosol, and stored at −80°C for enzyme activity analyses. The birds were then euthanized by cervical dislocation and exsanguinated. The livers and tibiae were then collected. The liver samples were excised, washed with 0.9% saline solution, frozen by direct plunging into liquid nitrogen, and stored at −80°C until analysis.

Productivity Performance

The birds were fed the 6 diets for a 7D adaptation period before any measurements were made (Xie et al., 2019). The numbers of total, broken, and shell-less eggs were recorded daily on a per replicate basis during the experimental period. All daily oviposited eggs were weighed on an individual basis. The feed intake (the difference between the added and refused feed amounts),

Table 1. Composition and nutrient levels in the basal diet of laying duck breeders (%, as fed basis).

Ingredients	Percentage (%)	Nutrient composition[2]	Level
Corn	52.30	AME, MJ/kg	10.45
Soybean meal	26.10	CP, %	18.0
Wheat bran	10.15	Ca, %	3.60
Limestone	8.70	Total P, %	0.60
Calcium hydrogen phosphate	1.30	Available P, %	0.35
DL-methionine	0.15	Total Lys, %	0.95
Salt	0.30	Total Met, %	0.40
Premix[1]	1.00	Total Met + Cys, %	0.70
Total	100	Se, mg/kg	0.11

[1]The premix provided the following (per kilogram of diet): vitamin A 12,000 IU, vitamin D_3 1,800 IU, vitamin E 26 IU, vitamin K 1.0 mg, vitamin B_1 3.0 mg, vitamin B_2 9.6 mg, vitamin B_6 6.0 mg, vitamin B_{12} 0.03 mg, choline 500 mg, D-calcium pantothenate 28.5 mg, folic acid 0.6 mg, biotin 0.15 mg, Fe 50 mg, Cu 10 mg, Mn 90 mg, Zn 90 mg, I 0.50 mg.
[2]Se content was measured in the mixed feed. Other nutrient levels are calculated values.

egg production, egg weight, egg mass (egg weight/bird/D), and feed conversion ratio (daily feed intake/daily egg mass) were calculated on a per replicate basis, and expressed as averages for the early-laying period (23–25 wk of age) with average daily egg production ranging from 50 to 80%, peak-laying period (26–49 wk of age) with average daily egg production by all ducks >80%, and the entire experimental period (23–49 wk of age).

Se Deposition and Malondialdehyde (MDA) Content in Egg

At 153 D of the experimental period (45 wk), 6 eggs were collected randomly from each replicate. Three eggs from each replicate (18 eggs/treatment) were used for Se content assay. The albumin plus yolk and shell were separated, and the albumin and yolk were freeze-dried for 72 h in a Christ ALPHA 1-2 LD plus freeze drying machine (Martin Christ, Osterode, Germany). The shells were washed by water, dried in an oven to a constant weight at 65°C, and smashed by an FW100 high speed grinder (Taisite Instrument Co., Ltd., Tianjin, China). Aliquots of all dried samples were weighed into digestion tubes and subjected to Se quantification based on fluorometric analysis (Cantor and Tarino, 1982). Se deposition efficiency in whole egg was expressed as daily Se deposition per egg/daily Se intake. The other 3 eggs from each replicate were used to detect yolk MDA content. Yolk (0.1 g) was obtained and weighed from each egg, and mixed with 0.9 mL anhydrous alcohol for 3 min. The mixture was centrifuged (12,000 × *g* for 10 min) to separate the supernatant, and 0.2 mL of the supernatant was obtained to determine the MDA content using kits (Jiancheng Bioengineering Institute, Nanjing, China).

Incubation Indices

During the peak-laying period, all birds were artificially inseminated twice (with a 3-D interval) with 100 μL of diluted fresh semen (diluted with 0.9% saline solution in a proportion of 1:1 vol/vol). The semen samples were collected from drakes belonging to the same breed. Fifty eggs per replicate (egg weight > 63 g, with no soft shells, cracks, dirtiness, or double yolks) were collected from the third through eighth day following the first insemination. All eggs were incubated in the same incubator (Bengbu Sanyuan Incubation Equipment Co., Ltd., Anhui, China) at 37.2°C to 38.0°C and 60 to 75% relative humidity for 28 D. Eggs were turned 12 times/D throughout the incubation period and sprayed with water once daily from the 15th D of incubation until they hatched. Egg fertility was checked by candling at the seventh day of incubation. After 28 D, the healthy hatched ducklings were counted and recorded, and eggs that failed to hatch were counted. Hatchability was calculated as hatchability of the fertile eggs. Healthy ducklings (clean and dry, free of deformities, and with bright eyes) were determined macroscopically as described by Tona et al. (2004). Twelve ducklings from each treatment group (2 ducklings from each replicate) at the hatching day were stunned, exsanguinated and samples of the liver were collected, rinsed quickly with PBS, snap frozen in liquid nitrogen, and stored at −80°C until analysis.

Tibial Quality

At termination of the study, the collected tibiae were trimmed of muscles and tendons, and weighed. Left tibiae were boiled in water for 6 min and defatted by soaking in diethyl ether for 96 h, and dried in an oven to a constant weight. The shaft length and width of the right tibiae were measured (Zhang and Coon, 1997). Besides, the right tibiae were used in examining the bone mineral density and mineral content at the Guangzhou Overseas Chinese Hospital with an X-ray osteodensitometer (Lunar Prodigy; General Electric Company, Fairfield, CT).

Chemical Analysis of Plasma, Erythrocyte, and Liver

The plasma activities of Gpx, total superoxide dismutase (**T-SOD**), and plasma contents of MDA were determined spectrophotometrically in duplicate using kits

(Jiancheng Bioengineering Institute) according to Huang et al. (2015).

Frozen liver (40 mg) was homogenized on ice in 4 mL of homogenization buffer (0.05 mol/L Tris-HCl, pH 7.4, 1 mmol/L EDTA, 0.25 mmom/L sucrose) with an Ultra-Turrax T8 (IKA, Staufen, Germany) for 5 s at 3,000 × *g*. The homogenate was centrifuged at 12,000 × *g* for 10 min at 4°C, and the supernatant was stored at −80°C. The concentrations of MDA and activities of T-SOD, Gpx, and total antioxidant capacity in the liver and the prepared erythrocyte cytosol of breeders, as well as in those of ducklings, were measured with kits (Jiancheng Bioengineering Institute).

Statistical Analysis

Data were statistically analyzed following the GLM method, and using the statistical software SAS version 9.1 (Copyright I 2002-2012; SAS Institute Inc., Cary, NC). Polynomial contrasts were used to identify the linear and quadratic responses to the dietary Se treatments. A broken linear regression model was employed to identify the optimal Se requirement. The broken linear regression model ($Y = \beta1 + \beta2 \times [\beta3 - Se]$) was followed, where ($\beta3 - Se$) = 0 for Se > β3 with Y as the dependent variable as a function of the dietary level of Se, β1 is the value of the dependent variable at the plateau, and β2 is the slope of the line. The Se level at the break point (β3) was considered the one providing maximum or minimum responses.

RESULTS

Productivity Performance

The laying performance indices of duck breeders aged 23 to 49 wk were unaffected by the tested dietary Se levels (Table 2).

Se Deposition and Yolk MDA Content

As shown in Table 3, Se content in the shell, yolk and albumin, and in the whole egg increased (linear, $P < 0.01$) as the dietary Se levels increased, and quadratic responses ($P < 0.05$) were observed. Se deposition efficiency was affected by dietary Se levels (linear, $P < 0.01$). Additionally, according to the broken linear model of Se deposition efficiency in relation to dietary Se levels, the highest Se deposition efficiency in the whole egg was obtained with the levels from 0.11 to 0.19 mg/kg Se. There were both linear ($P < 0.01$) and quadratic ($P < 0.05$) effects on yolk concentration of MDA in response to dietary Se levels.

Incubation Indices

The effects of dietary Se levels on the incubation indices of duck breeders are shown in Table 4. There were both linear ($P < 0.01$) and quadratic ($P < 0.05$) effects on fertility of set eggs in response to dietary Se levels, and the highest fertility of set eggs was obtained with 0.27 mg Se/kg diet. According to the broken linear model of egg fertility in relation to dietary Se levels, the optimal Se level was 0.27 mg/kg diet. Embryonic mortality and hatchability of fertile eggs on the hatching day were affected by dietary Se levels; the lowest embryonic mortality and highest hatchability were obtained with 0.27 mg Se/kg diet.

Tibial Quality

As shown in Table 5, the tibial quality indices of duck breeders aged 49 wk were unaffected by the tested dietary Se levels.

Table 2. Effects of dietary Se levels on laying performance of duck breeders aged 23 to 49 wk[1].

	Dietary Se (mg/kg)							*P*-value[2]
Item	0.11	0.19	0.27	0.35	0.43	0.51	SEM	Se
Early-laying period (50% < egg production < 80%, 23–25 wk of age)								
Feed intake (g/D)	142	142	142	141	141	142	0.34	NS
Egg production (%)	72.9	65.7	66.8	67.8	74.6	77.0	3.30	NS
Egg weight (g)	47.8	50.0	48.3	50.1	49.2	50.6	0.79	NS
Egg mass (g/D)	35.1	32.9	32.2	33.9	36.9	39.0	1.82	NS
Feed conversion (g/g)	4.19	4.45	4.51	4.27	3.92	3.71	0.20	NS
Peak-laying period (egg production >80%, 26–49 wk of age)								
Feed intake (g/D)	163	164	163	164	163	164	0.30	NS
Egg production (%)	88.9	87.9	86.8	88.2	87.7	89.3	1.24	NS
Egg weight (g)	62.5	62.4	62.6	63.1	62.9	63.4	0.32	NS
Egg mass (g/D)	56.1	55.1	55.1	55.4	55.1	56.5	0.68	NS
Feed conversion (g/g)	2.93	3.00	2.98	2.97	2.98	2.92	0.04	NS
Whole laying period (23–49 wk of age)								
Feed intake (g/D)	162	162	162	162	162	162	0.27	NS
Egg production (%)	87.9	86.5	85.5	87.0	86.9	88.5	1.25	NS
Egg weight (g)	61.6	61.7	61.6	62.3	62.1	62.6	0.32	NS
Egg mass (g/D)	54.9	53.7	53.7	54.1	53.9	55.4	0.66	NS
Feed conversion (g/g)	2.96	3.03	3.02	3.00	3.00	2.94	0.04	NS

[1]Data are means for n = 6 replicates (12 birds/replicate).
[2]Se: treatment effect; linear and quadratic effects were not significant; NS: no significance ($P > 0.05$).

Table 3. Effects of dietary Se levels on Se deposition in the whole egg and yolk MDA content of duck breeders aged 45 wk[1].

Item	Dietary Se (mg/kg)						SEM	*P*-value[2]		
	0.11	0.19	0.27	0.35	0.43	0.51		Se	L	Q
Daily Se intake (μg)	17.8	30.8	43.7	56.7	69.7	82.6	0.09	<0.01	<0.01	NS
Se content of yolk and albumin (μg)	6.77	14.8	20.7	21.2	22.5	26.9	1.05	<0.01	<0.01	<0.01
Se content of shell (μg)	6.17	7.62	8.73	9.68	9.34	9.38	0.53	<0.01	<0.01	<0.05
Se content in egg (μg)	12.9	22.4	29.4	30.9	31.9	36.3	1.29	<0.01	<0.01	<0.05
Se deposition efficiency (%)[3]	72.6	72.8	67.2	54.5	45.7	44.0	3.50	<0.01	<0.01	NS
Yolk MDA (nmol/mL)	131	127	110	109	82.7	38.8	12.2	<0.01	<0.01	<0.05

Abbreviation: MDA, malondialdehyde.
[1]Data are means for n = 6 replicates (3 eggs/replicate).
[2]Se: treatment effect; L: linear effect; Q: quadratic effect; NS: no significance ($P > 0.05$).
[3]Regression equation based on dietary Se level (mg/kg); broken linear equation: $Y = 72.6 + 98.7 \times (0.19 - Se)$, $Se \leq 0.19$; $Y = 72.6$, $Se > 0.19$; $R^2 = 0.96$, *P*-value < 0.01, yielded the optimized dietary Se value of 0.19 mg/kg.

Antioxidative Indices in Plasma, Erythrocyte, Liver, and Yolk of Duck Breeders

Antioxidative indices in plasma, erythrocyte, liver, and yolk of duck breeders are summarized in Table 6. There were both linear ($P < 0.01$) and quadratic ($P = 0.01$) effects on the activity of erythrocyte Gpx1, activities of T-SOD and Gpx1 in the liver in response to dietary Se levels. The plasma activity of Gpx3 increased (linear, $P < 0.01$) as the dietary Se level increased. Reciprocal quadratic ($P < 0.01$) changes in liver MDA concentration in response to dietary Se levels were evident; the lowest MDA concentration was observed with 0.43 mg Se/kg diet. According to the broken linear model of Gpx1 activity in erythrocytes and liver of duck breeders in relation to dietary Se levels, the optimal Se levels were 0.28 and 0.24 mg/kg.

Antioxidative Indices in Plasma, Erythrocytes, and Livers of Hatchlings

As shown in Table 7, erythrocyte activity of Gpx1 in newly hatched ducklings increased (linear, $P < 0.01$) with the increase in dietary Se levels of the breeder's diet. The activity of plasma Gpx3 and activities of liver Gpx1 in newly hatched ducklings were affected by dietary Se levels of the duck breeder's diet (linear, $P < 0.01$; quadratic, $P < 0.01$). According to the broken linear model of plasma activity of Gpx3 in newly hatched ducklings in relation to dietary Se levels of the breeder's diet, the optimal Se level was 0.30 mg/kg.

DISCUSSION

Some studies have shown that dietary Se from sodium selenite has no effect on egg production in both chicken breeders and layers (Leeson et al., 2008; Khashaba et al., 2009; Han et al., 2017). The findings of the present study confirm that the tested dietary Se levels from 0.11 to 0.51 mg/kg diet did not affect egg production of duck breeders aged 23 to 49 wk, but increased egg fertility and hatchability rates, with the best values obtained at 0.27 mg Se/kg. This agrees with the findings in pigeons reported by Wang et al. (2017), who suggested that dietary sodium selenite supplemented with both 0.5 and 1.0 mg/kg (containing 0.23 and 0.46 mg/kg Se) had no effect on egg production, whereas the use of 0.5 mg sodium selenite/kg (0.23 mg/kg Se) increased egg fertility. The results of these studies are contrary to the findings of Chen et al. (2015) in laying ducks, which indicated that dietary Se from 0.18 to 0.24 mg/kg is optimal for superior daily egg production during the early- to peak-laying periods. As noted earlier, inconsistencies in results and differences to what has been observed on egg production in the present study mainly reflect the difference in the animal models and Se deficiency level. Xiong et al. (2018) found that Gpx activity of oocytes and DNA integrity of cumulus cells significantly increased with supplemental 2 and 4 μg Se/mL of incubation medium during the in vitro maturation of oocyte, and high maturation efficiency and good quality of oocyte thereby enhanced subsequent embryonic development. A previous study demonstrated that low Se concentration in the follicular fluid may cause

Table 4. Effects of dietary Se levels on the incubation indices of duck breeders aged 40 wk[1].

Item	Dietary Se (mg/kg)						SEM	*P*-value[2]		
	0.11	0.19	0.27	0.35	0.43	0.51		Se	L	Q
Fertility of set eggs (%)[3]	74.5	79.5	88.3	85.6	87.2	86.3	2.57	<0.01	<0.01	<0.05
Embryonic mortality (%)	29.5	26.6	12.8	26.8	28.2	18.7	2.76	<0.01	NS	NS
Hatchability of fertile eggs (%)	69.5	70.1	83.9	69.4	71.8	80.2	2.71	<0.01	NS	NS
Healthy duckling (%)	96.5	99.4	98.5	98.8	98.5	99.5	0.87	NS	NS	NS
Duckling weight (g)	37.0	37.9	37.5	38.1	37.7	37.3	0.44	NS	NS	NS

[1]Data are means for n = 6 replicates (50 eggs/replicate).
[2]Se: treatment effect; L: linear effect; Q: quadratic effect; NS: no significance ($P > 0.05$).
[3]Regression equation based on dietary Se level (mg/kg); broken linear equation: $Y = 86.7 - 81.1 \times (0.27 - Se)$, $Se \leq 0.27$; $Y = 86.7$, $Se > 0.27$; $R^2 = 0.99$, *P*-value < 0.01, yielded the optimized dietary Se value of 0.27 mg/kg.

Table 5. Effects of dietary Se levels on tibial quality of duck breeders aged 49 wk[1].

	Dietary Se (mg/kg)							*P*-value[2]
Item	0.11	0.19	0.27	0.35	0.43	0.51	SEM	Se
Tibial fresh weight (g)	5.38	5.57	5.43	5.60	5.03	5.28	0.17	NS
Tibial dry weight (g)	3.30	3.36	3.32	3.35	3.12	3.29	0.10	NS
Tibial length (mm)	98.7	98.8	98.6	98.3	97.4	98.8	0.79	NS
Tibial width (mm)	6.04	5.91	5.92	6.08	5.92	5.82	0.10	NS
Mineral density (g/cm^3)	0.28	0.28	0.30	0.30	0.27	0.29	0.01	NS
Mineral content (g)	1.39	1.40	1.53	1.53	1.35	1.47	0.06	NS

[1]Data are means for n = 6 replicates (2 birds/replicate).
[2]Se: treatment effect; linear and quadratic effects were not significant; NS: no significance ($P > 0.05$).

unsolved infertility, and the antioxidative activity of Gpx in the follicular microenvironment may play an important role in gametogenesis and fertilization (Paszkowski et al., 1995). These could explain why the appropriate dietary Se level (0.27 mg/kg) increased egg fertilization and hatchability, with low embryonic mortality in the current study, which are close to the recommendation of 0.30 mg/kg Se for duck breeders in Commercial Poultry Nutrition (2004).

As expected, the Se content in the eggs of duck breeders including shell, yolk and albumin, increased with increasing dietary Se level in the current study; the highest Se level (0.51 mg/kg) increased Se content in the whole egg to 281% of the lowest content that occurred with the control (0.11 mg/kg). However, the deposition efficiency of Se in the whole egg exhibited a plateau from 0.11 to 0.19 mg/kg and decreased with the increase in dietary Se level from 0.19 to 0.51 mg/kg. It was construed that Se reserves in the body of ducks fed low-Se diets were easily mobilized to meet their requirement; thereby, the Se deposition efficiency in these eggs was greater than those fed with a high level of Se. Considering the Se deposition efficiency in eggs, it is suggested that using a Se level higher than 0.19 mg Se/kg diet is adequate for fulfilling the requirement of duck breeders.

Se is an indispensable structural component of the Gpx enzyme (Rotruck et al., 1973). The antioxidant effects of Se were shown to be mediated by Gpx activity, which eliminates potential damaging lipid hydrogen peroxides and plays a unique role in protecting cells against free radical-induced oxidative stress (Arthur, 2001), and MDA level that is negatively correlated with the Gpx activity (Ahmad et al., 2012). In this study, the linear increase of plasma Gpx3 activity and the decrease of hepatic MDA content and T-SOD activity with a quadratic pattern in response to the increase in dietary Se level from 0.11 to 0.51 mg/kg, along with the plateau from 0.24 to 0.51 mg/kg on erythrocyte and hepatic Gpx1 activity of duck breeders, indicate that supplementing the duck breeder diets with 0.24 to 0.51 mg/kg Se had a beneficial effect on improving their antioxidative capacity. This is consistent with the findings of Jing et al. (2015) in laying hens, which revealed that feeding hens with a diet containing 0.28 mg Se/kg led to higher plasma Gpx and T-SOD activity, and lower MDA content. In addition, in laying hens, previous studies indicated that serum GSH-Px activity was significantly increased when birds were fed diets containing 0.342 and 0.46 mg Se/kg (Invernizzi et al., 2013; Han et al., 2017). In the present study, the MDA content of yolk decreased as the dietary Se increased,

Table 6. Effects of dietary Se levels on antioxidative indices in plasma, erythrocytes, and liver of duck breeders aged 49 wk[1].

	Dietary Se (mg/kg)							*P*-value[3]		
Item[2]	0.11	0.19	0.27	0.35	0.43	0.51	SEM	Se	L	Q
Plasma										
T-SOD (U/mL)	17.7	17.3	17.5	19.7	17.4	17.9	1.25	NS	NS	NS
Gpx3 (U/mL)	313	509	497	489	493	594	28.3	<0.01	<0.01	NS
MDA(nmol/mL)	4.71	4.84	5.72	5.54	6.25	6.49	0.52	NS	NS	NS
Erythrocytes										
Gpx1 (U/mg protein)[4]	176	295	349	378	368	353	21.4	<0.01	<0.01	<0.05
Liver										
T-SOD (U/mg protein)	276	294	319	250	261	253	8.87	<0.01	<0.01	<0.05
Gpx1 (U/mg protein)[4]	40.6	288	449	411	427	485	12.0	<0.01	<0.01	<0.01
MDA(nmol/mg protein)	0.70	0.52	0.51	0.52	0.46	0.59	0.11	<0.05	NS	<0.01

[1]Data are means for n = 6 replicates (2 birds or 3 eggs/replicate).
[2]T-SOD: total superoxide dismutase; Gpx3: glutathione peroxidase 3; MDA: malondialdehyde; Gpx1: glutathione peroxidase 1.
[3]Se: treatment effect; L: linear effect; Q: quadratic effect; NS: no significance ($P > 0.05$).
[4]Regression equation based on dietary Se level (mg/kg); broken linear equation: Y (erythrocyte Gpx1) = 366.3 − 1081.2 × (0.28 − Se), Se ≤ 0.28; Y (erythrocyte Gpx1) = 366.3, Se > 0.28; $R^2 = 0.96$, *P*-value < 0.01. Y (liver Gpx1) = 443 − 3092.5 × (0.24 − Se), Se ≤ 0.24; Y (liver Gpx1) = 443, Se > 0.24; $R^2 = 0.98$, *P*-value < 0.01. These equations yielded the optimized dietary Se value of 0.28 and 0.24 mg/kg.

Table 7. Effects of dietary Se levels on antioxidative indices in plasma, erythrocytes, and liver of hatchlings[1].

	Dietary Se (mg/kg)							*P*-value[3]		
Item[2]	0.11	0.19	0.27	0.35	0.43	0.51	SEM	Se	L	Q
Plasma										
T-SOD (U/mL)	94.7	95.6	104	108	110	104	5.57	NS	NS	NS
Gpx3 (U/mL)[4]	452	772	890	1,022	987	1,026	53.2	<0.01	<0.01	<0.01
MDA(nmol/mL)	7.66	6.95	6.00	6.04	7.18	7.62	0.65	NS	NS	NS
Erythrocyte										
Gpx1 (U/mg protein)	184	244	231	252	243	273	13.3	<0.01	<0.01	NS
Liver										
T-SOD (U/mg protein)	567	557	566	507	537	539	14.4	NS	NS	NS
Gpx1 (U/mg protein)	167	275	407	364	420	374	11.1	<0.01	<0.01	<0.01
MDA(nmol/mg protein)	0.76	0.61	0.63	0.48	0.57	0.48	0.07	NS	NS	NS

[1]Data are means for n = 6 replicates (2 birds/replicate).
[2]T-SOD: total superoxide dismutase; Gpx3: glutathione peroxidase 3; MDA: malondialdehyde; Gpx1: glutathione peroxidase 1.
[3]Se: treatment effect; L: linear effect; Q: quadratic effect; NS: no significance ($P > 0.05$).
[4]Regression equation based on dietary Se level (mg/kg); broken linear equation: $Y = 1{,}012 - 2{,}738 \times (0.30 - Se)$, $Se \leq 0.30$; $Y = 1{,}012$, $Se > 0.30$; $R^2 = 0.97$, P-value < 0.01, yielded the optimized dietary Se value of 0.30 mg/kg.

accompanied with an increase in Se deposition in yolk and albumin, which indicated that the shelf life of the Se-enriched egg is probably longer than that of the Se-deficient egg for improving antioxidant status. With exception effects of the graded dietary Se levels on the antioxidant status of duck breeders and the effects shown on their eggs, there were obvious maternal transfer effects on the hatchlings in the present study, which were mainly reflected on plasma, erythrocyte, and hepatic Gpx activities, increasing with the increase in dietary Se level. The plasma Gpx3 activity reached a plateau pattern as Se increased from 0.30 to 0.51 mg/kg Se, which is in line with the egg fertility rate that occurred with Se increasing from 0.27 to 0.51 mg/kg. Maternal diets containing inadequate levels of Se led to reduced Se deposition in their eggs and the tissues of developing chicken embryos, as well as an imbalanced antioxidant defense system, increased accumulation of free radicals and reactive oxygen species, and greater oxidation of lipids and proteins, thereby leading to cell apoptosis, organ dysfunction, embryo death, and lower hatchability (Yuan et al., 2013; Xiao et al., 2016). With regard to Se deposition and antioxidant status, it is still unclear whether maternal dietary Se has a carryover effect to improve the growth performance of breeder's offsprings by enhancing the antioxidant status.

Se status is known to negatively correlate with bone metabolic turnover and positively with bone density in humans (Hoeg et al., 2012). The detrimental effects of Se deficiency were observed on bone microarchitecture in mice (Cao et al., 2012) and on bone mineral density in rats (Moreno-Reyes et al., 2001). In contrast to these results, this study showed no effects of dietary Se levels on bone quality in duck breeders, including the Se-deficient basal diet used here. In this study, the experimental birds were fed a commercial diet containing an adequate amount of Se during the growth period before the adaption of duck breeders, which may have led to long-term Se retention in the skeletal tissue and finally the apparent inconsistency with the former studies.

In conclusion, the dietary Se level did not affect egg production performance of Longyan duck breeders. The duck breeders fed with a 0.27 mg Se/kg diet had the highest egg fertility and hatchability, and dietary Se levels >0.19 mg/kg enhanced the antioxidant capacity of duck breeders and their offsprings. The regression analysis revealed that the optimal dietary Se requirements for maximum Se deposition efficiency in eggs, egg fertility, Gpx1 activity in erythrocytes and liver of duck breeders, and plasma Gpx3 activity in newly hatched ducklings were 0.19, 0.27, 0.28, 0.24, and 0.30 mg/kg, respectively. Finally, the diets of Longyan duck breeders should not contain less than 0.27 mg Se/kg to avoid reduced egg fertility and hatchability.

ACKNOWLEDGMENTS

This work was supported by the National Key Research and Development Program (Grant No. 2018YFD0501504), the Earmarked Fund for China Agriculture Research System (Grant No. CARS-42-13), the Pearl River Science and Technology Nova Program of Guangzhou (Grant No. 201710010159), the Key Project of the Science and Technology Program of Guangzhou City (Grant No. 201804020091 and 201904020001), the Modern Agricultural Industry Technology System Innovation Team of Guangdong Province (Grant No. 2019KJ137), the Special Fund for Scientific Innovation Strategy-Construction of High Level Academy of Agriculture Science (Grant No. R2017PY-QY008 and R2016PY-JG002), and the Science and Technology Program of Guangdong Province (Grant No. 2019A050505007).

Conflict of Interest Statement: The authors did not provide a conflict of interest statement.

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