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Effect of Dietary Supplemental Zinc on Laying Performance, Egg Quality, and Plasma Hormone Levels of Breeding Pigeons

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Abstract

This study aimed to evaluate the dietary zinc requirement of parental pigeons for better laying and reproductive performance, egg quality, sex hormones, and mineral content in eggs. A total of 160 pairs of healthy American Silver King pigeons were randomly assigned to five treatments of eight replicate cages each with four pairs of birds per cage, and fed a basal diet without zinc supplementation or the basal diet supplemented with 30, 60, 90, and 120 mg of zinc/kg ($ZnSO_4{\cdot}7H_2O$). The experiment lasted for 45 days, including two laying cycles. Results indicated the egg production rate ($P = 0.081$), egg shape index ($P = 0.038$), egg eggshell percentage ($P = 0.070$), and zinc and calcium contents ($P < 0.01$) tended to be affected or significantly affected by zinc addition. They increased quadratically with dietary zinc levels ($P < 0.05$). Besides, shell thickness ($P = 0.069$), plasma testosterone ($P = 0.008$), LH, and carbonic anhydrase contents ($P < 0.05$) tended to be affected or significantly affected by zinc addition. They increased linearly as dietary zinc level increased ($P < 0.05$). Compared with the control, 60 mg/kg zinc addition increased egg production rate, egg shape index, zinc and calcium contents in eggshell, and plasma testosterone concentration in pigeons ($P < 0.05$), and tended to increase the eggshell percentage ($P = 0.07$). Besides, supplemental 120 mg/kg zinc had higher shell thickness and LH content than control ($P < 0.05$), but had no difference with 60 mg/kg zinc addition. In conclusion, the supplementation of zinc at the level of 60 mg/kg to basal diet improved laying performance by increasing eggshell quality and sex hormone levels of breeding pigeons.

Keywords Zinc · Breeding Pigeons · Laying Performance · Eggshell Quality · Sex Hormone

Introduction

Pigeon eggs have long been recognized as the healthy animal-protein resources for humans because they are rich in proteins, lipids, amino acids, and minerals [1, 2]. With the improvement of our living standards, there is an increasing demand for pigeon eggs. It was reported that fewer sensitizations with pigeon egg were observed in children with egg allergy than duck egg and quail egg [3]. Notably, breeding pigeon eggs also provided the whole nutrient reservoir for embryo development. It is known that chicken, duck, goose, and quail are precocial birds that feed independently after birth. However, the fourth poultry, pigeons, are altricial birds that feed fully dependent on parents. Typically, pigeons were raised in pairs with a two-egg clutch, and there is a nearly 47-day breeding clutch interval, including eggs hatching and squabs feeding, resulting in a very lower egg production than other poultry [4]. These unique reproductive characteristics of breeding pigeons affect the laying performance and the squab yield, which immediately needs to be improved to meet consumer demand. Chang et al. [5, 6] found that dietary with 0.78% lysine or 1.20% calcium could improve the egg production rate of pigeons. Besides, the breeding female pigeons or laying hens with iodine-restricted diet reduced egg production, decreased egg hatchability, and retarded embryonic development compared with birds with iodine supplemental diet [7]. It was reported that dietary supplemented with 1 mg/kg sodium selenite displayed a better hatching performance of pigeons [8]. These studies show that maternal nutrition has an essential effect on embryonic development and postnatal growth in the offspring of poultry [9]. In addition, the eggshell of the pigeon eggs was thinner than that of other poultry eggs, including chicken eggs, duck eggs, goose eggs, and even quail eggs [10]. The thin

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eggshell in pigeon eggs could lead to higher egg breaking and bacterial contamination, which might reduce the shelf life and pose a threat to human health, decrease the hatching performance and squab production, and cause major economic losses [11, 12]. However, at present, there are few studies on improving the quality of pigeon eggs.

The National Research Council (NRC) has provided the nutritional requirements for chickens, ducks, gooses, and even Japanese quails, but not for pigeons. Some studies have been conducted on the requirements of energy and crude protein [13], amino acids [5], fatty acids [14], and mineral elements calcium [6] and phosphorus [15] in breeding pigeons. However, the requirements of trace elements are not clear. Zinc is one of the pivotal microelements necessary for normal animal function, including physical growth, muscle development, immunity function, reproduction, and hormone secretion [16]. It has been reported that supplementation of zinc in the diet can improve egg production, egg quality, and reproductive capacity by utilizing higher sex hormone concentration in broiler breeders and laying hens [17, 18]. In contrast, zinc deficiency in poultry breeders led to poor semen motility and fertilization ability, and reduced egg production performance with lower egg weight and egg quality [19]. It is well known that zinc is a component of the metalloenzyme carbonic anhydrase, which is involved in the eggshell formation [20]. Thus, zinc nutrition is crucial for egg production and eggshell quality in poultry egg industry. Additionally, maternal zinc nutrition affected embryonic development and postnatal growth performance through deposition of zinc in egg yolk of broiler breeders [21]. Based on egg production and hatchability, it has been recommended 35–50 mg/kg of zinc in the diet for breeder broilers, laying hens, and quails by NRC [22]. However, there is currently a lack of zinc nutritional recommendations for breeding pigeons to improve their laying and reproductive performances. Therefore, this study aimed to evaluate the supplementation of zinc requirements for better laying and reproductive performances, egg quality, sex hormones, and trace mineral content in eggs of parental pigeons.

Materials and Methods

Experimental Design, Birds, and Dietary Treatments

A total of 160 pairs of healthy American Silver King pigeons (1.5 years old) were randomly assigned to five dietary treatments, and fed a zinc-unsupplemented basal diet (22.93 mg of zinc/kg, Table 1) or the basal diet supplemented with 30, 60, 90, and 120 mg of zinc/kg. The zinc source is zinc sulfate heptahydrate ($ZnSO_4{\cdot}7H_2O$) in the form of reagent grade. Each group consisted of eight replicates and four cages per replicate, and a pair of breeding pigeons (one male and one female) with a two-egg clutch were raised in each cage. The size of each cage was 50 × 50 × 60 cm^2. Pigeons were fed a pellet diet based on corn, soybean meal, wheat, and sorghum. The composition and nutrient levels of the basal diet are shown in Table 1. Birds had free access to feed and water and maintained on a lighting cycle of 16-h light and 8-h darkness. The zinc content of drinking water was 0.128 mg/L, and it was not considered a significant dietary mineral source. The mean daily temperature was 22 ± 6 °C. The birds were provided with the basal pellet diet with 22.93 mg of zinc/kg for the 4 weeks of adaption period. The experimental period lasted for 45 days, including two laying cycles, and the two eggs from the second clutch were collected at 6 pm on the first day of laying for determination of laying performance, and egg quality (the two eggs from the first clutch were incubated and fed by parental pigeons to assess the growth performance of squabs).

Table 1 Composition and nutrient levels of the basal diet (air-dry basis)

Ingredients	Content (%)	Nutrient composition	
Corn	41.000	Metabolizable energy, MJ/kg	11.89
Soybean	13.125	Crude protein[4], %	15.85
Pea	17.420	Calcium, %	1.11
Wheat	12.800	Phosphorus[4], %	0.74
Sorghum	10.400	Lys, %	0.88
Soybean oil	0.700	Met, %	0.36
$CaHPO_4{\cdot}2H_2O$	2.087	Zinc[4], mg/kg	22.93
Limestone	1.350		
NaCl	0.400		
Cornstarch + zinc[1]	0.257		
Vitamin premix[2]	0.030		
Choline chloride	0.040		
Mineral premix[3]	0.101		
Lysine	0.160		
DL-Methionine	0.130		
Total	100		

[1]Zinc supplements replace equivalent weights of corn starch

[2]Complex vitamins are provided per kilogram of feed: VA 13500 IU, VD_3 3600 IU, VE 36 IU, VK_3 4.5 mg, VB_1 3.6 mg, VB_2 11.25 mg, D-pantothenic acid 16.5 mg, nicotinamide 39 mg, folic acid 2.1 mg, and biotin 0.24 mg

[3]Trace components are provided per kilogram of feed: iron 150 mg, copper 8 mg, manganese 65 mg, iodine 0.35 mg, selenium 0.25 mg

[4]Analyzed values and each value based on triplicate determinations, and other nutrients were calculated values

Laying Performance

The egg production rate (%) = the number of laying eggs/pigeon number × 100%, and the fertilization rate

(%) = the number of fertile eggs/the total number of laying eggs × 100% [23]. On the 3rd–5th day of incubation, an egg with red blood indicates fertilization when it was shined by flashlight; otherwise, it is not fertilized. The egg-laying interval was always accounted for from the day after the second egg laid in the first clutch to the day before the first egg laid in the subsequent clutch [24].

Sample Collection

On the 45th day of the experiment, the pigeons were treated with a 12-h feed deprivation, and eight pairs of breeding pigeons (eight male and eight female) from each treatment were randomly selected. The blood was collected aseptically from the wing portal vein, and the plasma was obtained after centrifuging at 3000 × g for 10 min at 4 °C and stored at − 20 °C for analysis of carbonic anhydrase and sex hormones. At the beginning of the second clutch, sixteen pigeon eggs from eight pairs of breeding pigeons in each treatment were randomly collected for determination of the egg quality.

Egg Quality Determination

Egg weight, albumen height, yolk color, and Haugh unit were measured using an egg quality analyzer (EMT7300, Robotmation, Japan). The yolk color is measured by Roche colorimetric fan scoring from 1 to 15. Eggshell strength was measured by an Egg Fore Reader (EFR-01, ORKA, Israel). Shell color was measured using an eggshell color tester (QCR, TSS, Britain), and expressed by reflectivity. Shell reflectivity, expressed as a percentage, is the amount of light that is reflected from the surface of an egg. The longitudinal diameter and transverse diameter were determined by a vernier caliper. The egg shape index was calculated from these measurements by dividing the transverse diameter by the longitudinal diameter. The eggshell percentage = eggshell weight/egg weight × 100%. After removing eggshell membranes, the eggshell thickness was measured using a digital micrometer at three points (blunt end, sharp end, and equator) and calculated by the average of three different thickness measurements of each egg. After egg quality determination, the yolk was separated from the albumen and placed in a sealed bag. The eggshell and egg yolk were frozen at − 20 °C for analysis of zinc concentration.

Plasma Carbonic Anhydrase and Sex Hormones

The plasma carbonic anhydrase and sex hormones, including testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), 17β-estradiol, and progesterone, were determined by the ELISA test (Bird ELISA Quantitation kit, Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China) following the manufacturer's protocol.

Zinc Contents in Diet, Eggshell, and Yolk and Calcium Content in Eggshell

Exactly 0.5 g of the basal diet and eggshells were weighed on the basis of dried material, and the 0.5 g of yolk weight was based on the fresh material. Then, they were digested with 10 mL HNO_3:$HClO_4$ (5:1 vol/vol) acid mixture in a 50-mL triangular flask at 160 °C using a sand heater until the solution was boiled to emit white smoke and become colorless. After cooling, the digested samples were diluted and transferred to 100 mL with deionized water in a volumetric flask. Zinc concentrations in diets, eggshell, and yolk and the calcium content in eggshell were determined by inductively coupled argon plasma spectroscopy (ICP 9000, Shimadzu, Japan) with zinc or calcium standard.

Statistical Analysis

The results were presented as means ± SEMs. All of the data were analyzed through the use of SPSS statistical software (version 17.0). Differences were evaluated by ANOVA. Comparisons between multiple groups were analyzed by post hoc Duncan's multiple range test. When a significant difference was observed by one-way ANOVA among five groups, the polynomial orthogonal contrasts were used to determine the linear and quadratic responses of dependent variables to dietary zinc supplementation. Differences were considered significant at $P < 0.05$ and a tendency was defined as $0.05 \le P < 0.10$.

Results

Effect of Dietary Supplemental Zinc on Laying Performance of Breeding Pigeons

The laying performance of breeding pigeons is shown in Table 2. Dietary supplemental zinc tended to affect the egg production rate ($P = 0.081$), but there was no significant difference observed in the egg weight, yolk weight, laying interval, and fertilization rate ($P > 0.05$). The egg production rate increased quadratically with dietary supplemental zinc levels ($P = 0.030$). The group with supplemental 60 mg/kg zinc had the maximum egg production rate, and the egg production rate was increased by 20.9% compared with the control group. Besides, the average daily feed intake of each bird from five groups (0, 30, 60, 90, and 120 mg/kg zinc supplementation) was 72.47, 72.16, 70.72, 73.38, and 72.70, respectively, and there is no significant difference among five groups (data are not shown in the table).

Table 2 Effect of dietary supplemental zinc on laying performance of breeding pigeons

Item	Egg production rate (%)[1]	Egg weight (g)[2]	Yolk weight (g)[2]	Laying interval (day)[1]	Fertilization rate (%)[1]
0	78.1^{b}	22.0	5.24	37.2	89.29
30	81.3^{b}	21.8	5.43	37.6	82.14
60	96.9^{a}	20.8	5.42	36.6	82.14
90	89.1^{ab}	21.8	5.73	36.3	87.50
120	84.7^{ab}	21.8	6.05	38.9	85.71
SEM	2.32	0.27	0.25	0.46	3.00
ANOVA	0.081	0.597	0.870	0.445	0.933
Linear	0.318	0.868	0.307	0.542	0.937
Quadratic	0.030	0.247	0.800	0.210	0.545

[1]Values represent means ± SEM ($n = 8$ replicates with 32 pairs of breeding pigeons per treatment); [2]Values represent means ± SEM (n = 8 replicates with 16 eggs per treatment). All values within a column followed by different superscript lowercase letters (a, b) differ significantly ($P < 0.05$)

Effect of Dietary Supplemental Zinc on Egg Quality of Breeding Pigeons

The egg quality of breeding pigeons is shown in Table 3. Dietary supplemental zinc significantly affected the egg shape index ($P = 0.038$), and the egg shape index increased quadratically with the increase of zinc levels ($P = 0.049$). Compared with the control group, dietary supplemental 30, 60, or 120 mg/kg zinc significantly increased the egg shape index ($P < 0.05$). The maximum egg shape index was observed in the groups with 60 mg/kg zinc addition. However, the albumen height, Haugh unit, yolk color, and the longitudinal and transverse diameters were not affected by zinc supplementation ($P > 0.05$).

Effect of Dietary Supplemental Zinc on Eggshell Quality of Breeding Pigeons

The eggshell quality of breeding pigeons is shown in Table 4. The supplemental zinc in the diet tended to affect the percentage of eggshells ($P = 0.07$) and shell thickness ($P = 0.069$). The percentage of eggshell increased quadratically ($P = 0.014$), and the shell thickness increased linearly ($P = 0.005$) as the dietary supplemental zinc level increased. Compared with the control group, dietary supplemental 60 mg/kg zinc tended to increase the eggshell percentage ($P = 0.07$), and dietary supplemental 120 mg/kg zinc significantly increased the shell thickness ($P < 0.05$). The shell strength and shell color were not affected by zinc supplementation in the diet ($P > 0.05$).

Effect of Dietary Supplemental Zinc on Plasma Hormone Levels of Breeding Pigeons

The levels of plasma hormone and carbonic anhydrase of breeding pigeons are shown in Table 5. The supplemental zinc in the diet significantly affected the testosterone content in plasma from male breeding pigeons ($P = 0.008$) and the contents of LH and CA in plasma from female breeding pigeons ($P < 0.05$). The testosterone concentration

Table 3 Effect of dietary supplemental zinc on egg quality of breeding pigeons

Zinc supplementation	Albumen height (mm)	Haugh unit	Yolk color (RCF)	Longitudinal diameter	Transverse diameter	Egg shape index
0	2.86	60.5	2.63	4.21	3.03	0.72^{b}
30	2.54	56.7	2.98	4.12	3.08	0.75^{a}
60	2.75	59.7	2.73	4.05	3.06	0.76^{a}
90	2.93	61.5	3.07	4.19	3.08	0.73^{ab}
120	3.02	63.3	2.87	4.16	3.11	0.75^{a}
SEM	0.091	1.09	0.110	0.022	0.015	0.004
P value	0.554	0.454	0.725	0.159	0.618	0.038
linear	0.269	0.182	0.488	0.801	0.158	0.100
Quadratic	0.318	0.292	0.602	0.076	0.977	0.049

Values represent means ± SEM ($n = 8$ replicates with 16 eggs per treatment) and values within a column followed by different superscript lowercase letters (a, b) differ significantly ($P < 0.05$). Egg shape index = transverse diameter/longitudinal diameter. *RCF* Roche colorimetric fan

Table 4 Effect of dietary supplemental zinc on eggshell quality of breeding pigeons

Zinc supplementation	Eggshell percentage (%)	Shell thickness (mm)	Shell strength (N)	Shell color (%)
0	10.66^{b}	0.26^{b}	1.10	63.4
30	10.99^{ab}	0.29^{ab}	1.11	64.6
60	12.04^{a}	0.30^{ab}	1.07	63.0
90	10.99^{ab}	0.31^{ab}	1.14	62.9
120	10.37^{b}	0.32^{a}	1.21	64.8
SEM	0.191	0.007	0.027	0.340
P value	0.070	0.069	0.595	0.221
linear	0.658	0.005	0.234	0.343
Quadratic	0.014	0.607	0.350	0.476

Values represent means ± SEM ($n = 8$ replicates with 16 eggs per treatment) and values within a column followed by different superscript lowercase letters (a, b) differ significantly ($P < 0.05$). Eggshell percentage = eggshell weight/egg weight × 100%

Table 5 Effect of dietary supplemental zinc on plasma hormone and carbonic anhydrase levels of breeding pigeons

Zinc supplementation	Testosterone (pg/mL)	FSH (mIU/mL)	17β-Estradiol (pg/mL)	PROG (pg/mL)	LH (mIU/mL)	CA (ng/mL)
0	206.71^{b}	5.91	135.1	1.85	1.34^{b}	7.02^{b}
30	269.55^{a}	5.30	117.0	1.45	1.34^{b}	7.08^{b}
60	253.55^{a}	5.25	123.1	1.59	1.73^{ab}	8.31^{b}
90	283.37^{a}	5.60	124.8	1.58	1.45^{b}	10.30^{ab}
120	270.05^{a}	6.03	145.7	1.87	1.99^{a}	13.23^{a}
SEM	7.66	0.21	3.79	0.096	0.084	0.596
P value	0.008	0.710	0.108	0.588	0.032	0.001
Linear	0.005	0.713	0.262	0.817	0.013	< 0.001
Quadratic	0.062	0.180	0.021	0.145	0.523	0.102

The plasma testosterone level was from male breeding pigeons, and the other plasma hormone and carbonic anhydrase levels were from female breeding pigeons. Values represent means ± SEM ($n = 8$ pigeons per treatment) and values within a column followed by different superscript lowercase letters (a, b) differ significantly ($P < 0.05$). *CA* carbonic anhydrase, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *PROG* progesterone

increased linearly ($P = 0.005$) and tended to increase quadratically ($P = 0.062$) as the dietary supplemental zinc level increased. The contents of LH and CA in plasma from female breeding pigeons increased linearly as dietary supplemental zinc levels increased ($P < 0.05$). Compared with the control group, dietary supplemental zinc significantly increased the plasma testosterone concentration ($P < 0.05$), but there is no significant difference among supplemental 30, 60, 90, or 120 mg/kg zinc in the diet. Dietary supplemental 120 mg/kg zinc had higher the levels of LH and CA than the control group ($P < 0.05$), but there is no significant difference in LH levels between the group supplemental 120 mg/kg zinc and the group supplemental 60 mg/kg zinc ($P > 0.05$). The FSH, 17β-estradiol, and PROG contents in plasma from female breeding pigeons were not affected by zinc supplementation ($P > 0.05$).

Effect of Dietary Supplemental Zinc on the Zinc Contents in Eggshell and Yolk and the Calcium Content in Eggshell of Breeding Pigeons

The zinc contents in eggshell and yolk and the calcium content in eggshell of breeding pigeons are shown in Table 6. The zinc and calcium contents in eggshells were significantly affected by the dietary supplemental zinc levels ($P < 0.01$). The linear and quadratic responses were observed in the zinc and calcium contents of eggshells ($P < 0.05$). Compared with the control group, dietary supplemental 60 and 90 mg/kg zinc significantly increased the zinc content in eggshell ($P < 0.01$), and dietary supplemental different levels of zinc increased the calcium content in eggshell ($P < 0.001$). The egg yolk zinc content was not affected by zinc supplementation in the diet ($P > 0.05$).

Table 6 Effect of dietary supplemental zinc on the zinc content of eggshell and yolk and the calcium content of eggshell of breeding pigeons

Zinc supplementation	Eggshell zinc content (μg/g)[1]	Egg yolk zinc content (μg/g)[1]	Eggshell calcium content (%)
0	2.97^{b}	38.03	27.71^{b}
30	9.25^{b}	33.86	31.69^{a}
60	20.70^{a}	29.34	31.91^{a}
90	25.76^{a}	33.89	32.05^{a}
120	9.12^{b}	41.86	31.68^{a}
SEM	2.15	2.06	0.76
P value	0.001	0.182	<0.001
linear	0.021	0.612	<0.001
Quadratic	0.001	0.029	<0.001

Values represent means ± SEM ($n = 8$ replicates with 16 eggs per treatment) and values within a column followed by different superscript lowercase letters (a, b) differ significantly ($P < 0.05$)

[1]The zinc contents in eggshell and yolk were calculated on the base of wet weight of eggshell or yolk

Discussion

It is known that the micromineral zinc plays an important role in improving the reproductive efficiency in livestock, birds, and humans [25, 26]. Other authors also observed the increases in egg production of various bird species when zinc was supplemented at the levels of 30, 45, 65, 67, and 160 mg/kg zinc in diets fed to laying hens [27], laying ducks [28], duck breeders [29], broiler breeder hens [30], and Japanese quails [31], respectively. There are no relevant published studies about the effect of zinc on the reproductive performance in breeding pigeons. Our current study showed that the egg production of breeding pigeons was affected by dietary supplemental zinc, and it was maximum when zinc was supplemented at 60 mg/kg zinc in diets. It is reported that maternal zinc nutritional status could influence the normal embryo development through the epigenetic mechanism [19]. And the egg yolk is the primary mineral source for the development of chicken embryo, which is abundant with zinc, copper, manganese, and iron [32]. In the present study, yolk zinc deposition was not affected by dietary zinc supplementation. The possible reason was that some eggs were laid in the earlier morning, and some might be in the late afternoon, and the time difference may cause different degrees of embryonic development. It is known that the yolk is a short-term storage site for trace minerals, and it has the ability to regulate the export of trace minerals through the capillary system to the embryo during development. Therefore, the zinc level in yolk was not affected by the increasing levels of zinc in diets. Besides, the zinc content in eggshells was significantly increased with the increase levels of zinc, but declined in the group supplemental 120 mg/kg of zinc. Calcium, especially in the presence of phytate, may interfere with zinc absorption [33]. Significantly, the eggshell calcium content increased with the increase levels of dietary zinc. The greater zinc supplementation might enlarge the negative effect of the calcium and phytate in basal diet due to the formation of an insoluble complex [34, 35], leading to the decrease of zinc absorption in the gastrointestinal tract and final deposition in eggshell.

Eggshell quality is one of the most critical problems in the egg industry, and the cracking of eggshells influence the economic profitability of egg production and hatchability [36, 37]. Therefore, the increase in egg production rate may have occurred due to the improved eggshell quality. As it is, our current study also demonstrated that dietary supplemental zinc resulted in higher-quality eggs than the control group, as evidenced by the improved eggshell percentage and shell thickness. It was reported that the eggshell thickness was considered an important physical barrier against bacterial trans-shell contamination [10]. And the highest eggshell percentage was observed in breeding pigeons fed with a level of 60 mg/kg zinc in the diet. These discoveries were in concurrence with other published studies, which correlated inorganic or organic zinc supplementation with improvement in eggshell quality, including breaking strength and eggshell percentage [38, 39] and eggshell thickness [40] of laying hens. Additionally, the egg geometry is also essential indicators of eggshell quality and hatchability, and is typically determined by the egg shape index, as defined by the ratio of egg transverse diameter to longitudinal diameter [41]. In this research, supplemental different levels of zinc could increase the egg shape index, which are in line with the results of Gholizadeh et al. [42], who found that dietary supplemental zinc oxide alleviated the decline of egg shape index of laying hens during heat-stress conditions. Besides, we found the supplementation of 60 mg/kg of zinc has the maximum value of egg shape index, but has the minimum value of shell strength. Gervais et al. [43] found that the rounder eggs from White Leghorns tended to be more resistant than more elongated eggs to breakage by analyzing the genetic correlation between eggshell breaking strength and shape index. In contrast, Duman et al. [44] showed that the shape index of hen eggs did not affect breaking strength and there was no significant correlation between egg shape index and breaking strength. The possible reason for the differences in the above results may be related to the different breed and age of laying hens. A recent study found that the quail eggs with a shape index of more than 78% contribute to increased hatchability efficiency [45]. It is necessary to determine the hatchability of the pigeon eggs in our future study.

Zinc affects egg formation through regulating the activity of carbonic anhydrase in oviduct epithelium [46]. Carbonic anhydrase is a zinc-containing enzyme that plays an essential role in the synthesis of eggshell calcium carbonate of breeders by supplementation of carbonic ion [20]. It is likely that zinc may have led to a higher activity of plasma carbonic anhydrase and then led to calcium deposition in eggshells and an improvement in eggshell thickness. It is a truth that the concentration of plasma carbonic anhydrase and the content of eggshell calcium were upregulated by dietary zinc supplementation in the present study. However, the exact mechanisms of how zinc affects calcium deposition by regulating carbonic anhydrase need to be demonstrated in further research.

In laying hens, follicular development, maturation, and ovulation are mainly regulated by gonadotropins, for example, FSH and LH [47]. Accordingly, the level of plasma hormone, to some extent, was usually considered a sensitive indicator of egg production performance of hens [48]. It has also been demonstrated that the surge in LH release promotes the large follicles with fully mature oocytes to ovulate [49, 50]. Zinc plays a vital role in the synthesis of sex hormones, and the level of LH in female breeding pigeons was increased by 60 or 120 mg/kg dietary zinc supplementation in the present study, which likely promoted ovulation. This increased LH level may have led to the observed improvement in egg production rate in the appropriate amount of zinc supplementation in present study. In addition, the level of testosterone in male breeding pigeon was higher in different levels of zinc supplementation groups, and these results coincide with the earlier report [26]. It was reported that testosterone could mimic the action of LH on oocyte maturation, and zinc mediates the action of testosterone in acting as a downstream effector of LH on oocyte maturation in zebrafish [51]. Zinc is also essential for testosterone metabolism, the production of healthy sperm, and the increase of sperm motility [52, 53]. It was attributed that higher testosterone and better semen quality might be the reason for increased fertility [54]. However, the fertility rate of pigeon eggs was not affected by dietary zinc supplementation in the present study. The semen quality, including sperm production and motility, was not analyzed in the present study due to the difficulty of collecting the pigeon semen.

In conclusion, the supplemental zinc to breeding pigeons improved egg production rate and eggshell quality by increasing plasma sex hormone levels, egg shape index, eggshell percentage, shell thickness, and eggshell calcium content. The supplementation of zinc in the form of inorganic $ZnSO_4$ at the level of 60 mg/kg to the basal diet had better laying performance including egg production rate and eggshell quality of breeding pigeons. Overall, this study would be necessary for developing and formulating the zinc requirement standard of the breeding pigeons based on the laying performance in the future.

Author Contribution Yuxin Shao analyzed the data and wrote the manuscript. Xing Li and Shaohua Du carried out the animal experiments. Xiaoshan Sun collected the sample and material. Yangyang Wang and Dongdong Zhao prepared all the tables. Zheng Wang designed and supervised the experiment. All authors read and approved the final manuscript.

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Data Availability The data of this study will be made available on reasonable request.

The study was carried out in accordance with the guidelines set by the Animal Care and Use Committee (permit number: SYXK-2017–0005) of the Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences (IAHVM-BAAFS), Beijing, China. The protocols were approved by the Animal Care and Use Committee of IAHVM-BAAFS.

Declarations

Competing interests The authors declare no competing interests.

Conflict of Interest The authors declare no competing interests.

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