Biological Trace Element Research (2020) 196:597–606
https://doi.org/10.1007/s12011-019-01927-9

Effect of Dietary Zinc Level on Egg Production Performance and Eggshell Quality Characteristics in Laying Duck Breeders in Furnished Cage System

Liang Huang [1] · Jiajia Shen [1] · Yan Feng [1] · Daquan Li [2] · Wence Wang [1] · Lin Yang [1] · Yongwen Zhu [1]

Received: 6 June 2019 / Accepted: 3 October 2019 / Published online: 20 January 2020

Abstract

In order to investigate the effect of dietary Zn levels on laying performance, eggshell quality, and eggshell microstructure in Muscovy duck breeders under furnished cages. Firstly, the effects of age (35 weeks vs 40 weeks) and rearing system (littered floor vs furnished cage) on eggshell quality of laying duck breeders were studied (Exp. 1). Then, a total of 324 30-week-old Muscovy duck breeders were allotted into 3 dietary Zn groups with 6 replicates (18 ducks per replicate), including 0 mg Zn/kg (control-Zn group, C-Zn), 40 mg Zn/kg (normal-Zn group, N-Zn), and 140 mg Zn/kg (high-Zn group, H-Zn). The experimental period for 6 weeks was divided into 3 periods of 30–32, 32–34, and 34–36 weeks of age (Exp. 2). In Exp. 1, duck breeder eggs in the furnished cage system had lower the average shell thickness than birds in the littered floor system at 40 weeks of age ($P < 0.05$), not at 35 weeks of age. In Exp. 2, N-Zn and H-Zn groups had greater egg weight, egg production, and egg to feed ratio of duck breeders than C-Zn group ($P < 0.05$). Additionally, H-Zn group had higher laying rate, qualified egg ratio, and Haugh unit as well as lower mammillary cone width than C-Zn group ($P < 0.05$), with no differences between C-Zn and N-Zn groups ($P > 0.05$). Diet supplemented with 140 mg Zn/kg increased shell thickness and palisade layer thickness of duck breeders at 36 weeks of age ($P < 0.05$), but not at 32 and 34 weeks of age. In conclusion, diets with 40 or 140 mg Zn/kg improved egg production performance and egg quality of laying duck breeders during 30–36 weeks of age in a furnished cage system. Dietary supplementation of 140 mg Zn/kg level increased the ultrastructural palisade layer thickness contributing to greater eggshell thickness of duck breeders at 36 weeks of age.

Keywords Duck breeders · Eggshell microstructure · Eggshell thickness · Zinc

Introduction

With the development of duck industry the traditional free-range rearing system resulted in the low nutrient efficiency high incidence of diseases and severe water pollution of wastage in duck production [1, 2]. The duck housing system is being gradually shifted from the non-caged system to the caged system to ensure the better health status and maximal economic benefit in China. However one major issue was the fact that the caged system provided a restricted physical and psychological space and prevented birds from performing natural behaviors compared with the non-caged system [3].

Liang Huang and Jiajia Shen contributed equally to this work.

✉ Lin Yang
ylin898@scau.edu.cn

✉ Yongwen Zhu
408034085@qq.com

Liang Huang
2824576@qq.com

Jiajia Shen
10457146@qq.com

Yan Feng
85742361@qq.com

Daquan Li
10424528@qq.com

Wence Wang
93608449@qq.com

[1] Guangdong Provincial Key Laboratory of Animal Nutrition and Regulation, College of Animal Science, South China Agricultural University, Guangzhou, China

[2] Daping Duck Breeding Farm, WENS Group, Yunfu, China

Moreover the caged system exerted some negative effects on the productivity of laying birds due to limited movement such as poor bone strength [4] and egg quality [5, 6]. Poor eggshell quality characteristics could reduce the shelf life and safety of eggs and egg products [7]. For example the decreased eggshell thickness could increase the risk of the bacterial contamination in hatching eggs [8] and then impair hatchability performance of laying breeders [9]. However there is limited systematic information available on eggshell quality of duck breeders in alternative furnished cage system. Some researchers have proposed that the effects of different housing systems on eggshell quality were also dependent on the layer age [10, 11]. Therefore we firstly investigated the effects of bird age and rearing system (floor pen vs furnished cage) on eggshell quality of laying duck breeders

Zinc (Zn) as a component of carbonic anhydase (CA) enzyme plays a role in eggshell formation [11, 12]. Many studies have showed that dietary Zn supplementation enhanced eggshell quality by increasing eggshell thickness and strength of hen layers and duck layers [13, 14]. Dietary Zn requirement recommendations for Peking duck breeders in NRC (1994) [15] and Muscovy duck breeders in INRA (1989) [16] were sourced from the data based on the non-caged systems. However, caged system that allows less movement of birds could influence feed intake and energy expenditure of birds [17]. Thus, nutrient requirements should be modified as with the changes of physical activity and energy intake of birds under the different housing systems [6]. So far, there has been little research on dietary Zn nutrition for improving eggshell quality of duck breeders reared in furnished cage system. Therefore, the effects of dietary Zn levels on laying performance, egg quality, and eggshell microstructure were investigated in Muscovy duck breeders under furnished cages in the present study.

Materials and Methods

Animals and Diets

All procedures of our experiments were approved by animal care and welfare committee institute of South China Agricultural University. Firstly, the eggshell quality of laying Muscovy duck breeders reared in two rearing technologies, a cage-free system and a furnished cage system, was compared (Exp. 1). Sixty Muscovy ducklings were selected and housed in the plastic floor system (from 0 to 12 weeks of age) and caged-free littered system (from 13 to 27 weeks of age) at the early laying period. After that, sixty laying duck breeders were divided into two groups consisting of thirty birds, which were placed into littered floor system (LF) and furnished cage system (FC), respectively. The littered system had an indoor and outside dirt lot, which both had perches (15 cm^2 of area per duck) and nest box (4 ducks per nest). The floor in the indoor area was covered with wood shavings as a litter material. The outside area without grass or forage had a shelter and was enclosed by wire fences to keep out predators. A total of seven and eight ducks per square meter were provided in the indoor and outside areas, respectively. The furnished cage met the requirement of the EU Directive 1999/74/EC. The furnished cage units were made of a wire mesh floor, galvanized metal partitions between the cages, and full-opening fronts at each long side of the cages consisting of spaced horizontal bars. All laying duck breeders' ducks were fed restrictively with a commercial feed at the same nutritional level (11.82 MJ ME/kg, 180 g CP/kg, 8.0 g lysine/kg, 7.2 g methionine + cysteine/kg, 33.0 g Ca/kg, 3.8 g available P/kg). Water was available ad libitum. The droppings were automatically removed on manure belts. The 20 eggs were collected from the laying birds between at the end of 35 and 40 weeks of age, respectively. A total of 40 eggs from each rearing system were analyzed for eggshell quality. In Exp. 2, a total of 370 26-week-old Muscovy duck breeders obtained from a commercial duck breeder farm (WENS Group, Yunfu, Guangdong) were housed in furnished cages for 4 weeks of an adaptation period. During the early laying period, all ducks were fed restrictively with a commercial feed at the nutritional level (11.32 MJ ME/kg, 180 g CP/kg, 7.0 g lysine/kg, 7.2 g methionine + cysteine/kg, 24.0 g Ca/kg, 3.8 g available P/kg, 40 mg Zn/kg). Water was provided ad libitum. At 30 weeks of age, 324 laying duck breeders were selected, balanced for laying rate, and then randomly allotted into 3 dietary Zn groups with 6 replicates (18 ducks per replicate). The experimental diets included base diet without Zn supplementation (control-Zn group, C-Zn), basal diet + 40 mg Zn/kg (normal-Zn group, N-Zn), and basal diet + 140 mg Zn/kg (high-Zn group, H-Zn) as $ZnSO_4.7H_2O$ source. The basal diet was formulated to meet or exceed the nutritional requirements of laying duck breeders except Zn. The composition and nutrient level of the basal diet were shown in Table 1. The analyzed values of Zn contents in C-Zn, N-Zn, and H-Zn diets were 29.2, 63.4, and 163.4 mg/kg, respectively. The experimental period lasting for 6 weeks was divided into 3 periods of 30–32, 32–34, and 34–36 weeks of age. All ducks had diets restrictively and access to water ad libitum according to the operation manual and guideline of Muscovy duck breeders. The ducks received 16 h of daily lighting from 04:30 am to 20:30 pm. Room temperature and humidity were controlled by the air conditioner and recorded daily. Manure was removed through an automatic belt system daily. All eggs were collected from each replicate daily and egg production (number of total laid eggs, defective eggs, and average qualified egg weight) were recorded daily. Feed consumption and

Table 1 Composition and nutrient levels of the basal diets for laying duck breeders during 30–36 weeks of age (as-fed basis)

Item (%)	Laying period
Corn	51.67
Soybean meal	17.70
Corn gluten meal	7.75
Wheat middlings	8.97
Lard	1.84
Dicalcium phosphate	1.80
Limestone	8.50
Sodium chloride	0.30
DL-Methionine	0.27
L-lysine·HCl	0.20
Vitamin and mineral premix[1]	1.00
Total	100.00
Nutrient composition	
Calculated value (%)	
ME, MJ/kg	11.63
Crude protein	18.51
Calcium	3.70
Total phosphate	0.60
Non-phytin phosphorus	0.44
Lysine	0.91
Methionine	0.57
Methionine + cysteine	0.84
Zinc[2]	29.2

[1] Provided per kilogram of diet without Zn addition: vitamin A, 5000 IU; vitamin D_3, 800 IU; vitamin E, 20 IU; thiamine, 2.0 mg; riboflavin, 15 mg; pyridoxine, 4.0 mg; vitamin B_{12}, 0.02 mg; calcium pantothenate, 10 mg; folate, 0.15 mg; niacin, 60 mg; biotin, 0.20 mg; choline (choline chloride), 1500 mg; Cu ($CuSO_4 \cdot 5H_2O$), 8 mg; Fe ($FeSO_4 \cdot 7H_2O$), 80 mg; Mn ($MnSO_4 \cdot H_2O$), 100 mg; Se ($NaSeO_3$), 0.3 mg; I (KI), 0.4 mg

[2] Analyzed values based on triplicate determinations

egg weight were measured weekly. Feed intake was calculated by dividing total feed consumed by numbers of ducks per replicate per day. Four eggs based on the average egg weight each replicate were collected at the last day of each period for the measurements of egg quality and eggshell microstructure.

Sample Analyses

The Zn concentrations in diets were measured using atomic absorption spectrophotometry (model Shimadzu AA-6800, Tokyo, Japan). At the end of 32, 34, and 36 weeks of age, four eggs per replicate were used to measure the related indices of egg quality. The color of the yolk and albumen height from the collected eggs were determined using an egg multitester (model EMT-5200, Touhoku Rhythm Ltd., Tokyo, Japan). Haugh unit was calculated based on egg weight and albumen height (Haugh, 1937). Shell strength was determined with an Egg Force Reader (model EFR-01, Orka, Ramat HaSharon, Israel). Eggshell was separated from albumen and yolk, washed to remove residual albumen, and cleaned by a paper towel and then weighed. Eggshell thickness was measured without inner and outer shell membranes at the blunt end (bottom), sharp end (top), and middle using a peacock dial pipe gauge (model P-1, Ozaki MFG Ltd., Tokyo, Japan). Shell thickness was calculated as the average thickness at the three points of eggshell. Eggshell microstructures were measured by scanning electron microscopy as described by Berwanger et al. (2018) [17]. Average values of the mammillary cone width and the thickness of mammillary layer, palisade layer, and eggshell membrane were estimated from 3 measurements (μm) in each photograph.

Statistical Analysis

Data were analyzed by 2-way ANOVA using the PROC GLM procedure of the SAS 9.2 (SAS Inst. Inc., Cary, NC). The models included rearing system and age, and their interactions for Exp. 1, as well as dietary Zn, period, and their interactions for Exp. 2. The replicate served as the experimental unit. Differences among means were tested by the LSD method, and statistical significance was set at $P \leq 0.05$.

Results

Eggshell Quality

Shell weight, shell strength, and shell thickness were affected by age ($P < 0.01$), not by rearing system ($P > 0.05$). The eggs from laying duck breeders at 40 weeks of age had higher shell weight and weaker shell strength and thickness than those from birds at 35 weeks of age. The interaction between age and system affected the average shell thickness ($P = 0.05$) and had no effect on other measured indices ($P > 0.05$). The rearing system did not influence the average shell thickness of duck breeder eggs at 35 weeks of age, whereas FC system had a lower average shell thickness of duck breeder eggs at 40 weeks of age compared with the LF system (Table 2).

Egg Production Performance

Egg weight, laying rate, egg production, egg to feed ratio, and qualified eggs ratio were affected by age ($P < 0.0001$) and dietary Zn ($P < 0.05$), not by their interaction ($P > 0.13$). Egg weight, laying rate, egg production, egg to feed ratio, and qualified eggs ratio were increased as age and dietary Zn level increased ($P < 0.04$). Egg weight, laying rate, egg production, egg to feed ratio, and qualified eggs ratio of duck breeders during 32–34 weeks and 34–36 weeks of age were greater than birds during 30–32 weeks of age ($P < 0.0001$),

Table 2 Effects of age and rearing system on eggshell quality of laying duck breeders at 35 and 40 weeks of age

Age (week)	System	Shell weight (g/egg)	Shell strength (kg/N)	Shell thickness (cm)			
				Top	Middle	Bottom	Average
35[1]	LS	9.51	5.77	0.423	0.427	0.418	0.423[a]
	FC	9.31	6.19	0.421	0.431	0.42	0.424[a]
40[1]	LS	10.43	4.37	0.347	0.367	0.359	0.358[b]
	FC	11.28	4.15	0.315	0.336	0.329	0.329[c]
	SEM	0.29	0.31	0.01	0.01	0.01	0.008
System[2]	LS	9.97	5.07	0.39	0.397	0.389	0.391
	FC	10.30	5.17	0.37	0.384	0.375	0.377
	SEM	0.27	0.25	0.01	0.01	0.01	0.006
Age[3]	35 weeks	9.41	5.98	0.42	0.429	0.419	0.424
	40 weeks	10.86	4.26	0.33	0.352	0.344	0.344
	SEM	0.23	0.20	0.01	0.01	0.01	0.006
P value	System	< 0.0001	< 0.001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Age	0.32	0.46	0.86	0.28	0.22	0.27
	Age*System	0.59	0.10	0.17	0.17	0.20	0.05

[a–c] Means within a column lacking a common superscript differ ($P < 0.05$)

[1] Values represented the means of 20 replicates ($n = 20$)

[2] Values represented the means of 40 replicates ($n = 40$)

[3] Values represented the means of 40 replicates ($n = 40$)

with no differences between the periods of 32–34 weeks and 34–36 weeks of age ($P > 0.05$). Compared with C-Zn group, N-Zn and H-Zn groups increased egg weight, egg production, and egg to feed ratio of duck breeders ($P < 0.02$), with no differences between N-Zn and H-Zn groups ($P > 0.36$). Duck breeder from H-Zn group had higher laying rate and qualified egg ratio than C-Zn group ($P < 0.01$), and no differences were observed between C-Zn and N-Zn groups as well as N-Zn and H-Zn groups ($P > 0.24$) (Table 3).

Egg Quality

Shell thickness, Haugh unit, and yolk color were affected by duck age ($P < 0.03$). Dietary Zn and the interaction between age and dietary Zn influenced ($P < 0.04$) Haugh unit and shell thickness, respectively. Age, dietary Zn, and their interaction had no effects ($P > 0.05$) on the shell weight and shell strength of duck breeders. Eggs from ducks at 34 weeks old had higher Haugh unit and lower shell thickness than birds at 32 and 36 weeks old ($P < 0.03$), while egg yolk color from ducks at 34 and 36 weeks old was higher than birds at 32 weeks old ($P < 0.01$). Haugh unit in H-Zn group was higher ($P < 0.02$) than C-Zn group, with no differences between C-Zn and N-Zn groups. Dietary Zn level had no effect on shell thickness of duck breeders at 32 and 34 weeks of age ($P > 0.27$), whereas dietary supplementation of 40 and 140 mg Zn/kg increased shell thickness of duck breeders at 36 weeks of age compared with the control group ($P < 0.05$) (Table 4).

Eggshell Microstructure

The mammillary cone width and thickness were affected ($P < 0.02$) by bird age. Dietary Zn level had an effect ($P < 0.02$) on mammillary cone width and did not affect ($P > 0.05$) mammillary cone width, palisade layer thickness, and membrane thickness. The interaction between age and dietary Zn influenced ($P = 0.05$) palisade layer thickness of ducks. Eggs from ducks at 32 and 34 weeks old had greater mammillary cone width than birds at 36 weeks old, while H-Zn group had a lower mammillary cone width and a greater membrane cross-linking structure than C-Zn and N-Zn groups ($P < 0.01$; Figs. 1 and 2). Dietary Zn supplementation had no effect on palisade layer thickness of duck breeders at 32 and 34 weeks of age ($P > 0.48$), whereas H-Zn group increased palisade layer thickness of duck breeders at 36 weeks of age compared with C-Zn group ($P < 0.04$), with no differences between C-Zn and N-Zn groups ($P > 0.72$) (Table 5).

Discussion

Broken egg problem is a large financial loss to the poultry industry. The eggshell quality is one of the most important

Table 3 Effects of age and dietary Zn on egg production performance of duck breeders at 30 to 36 weeks of age under furnished cage system

Period (week)	Dietary Zn (mg/kg)	Egg weight (g)	Laying rate (%)	Egg production (g/bird/d)	Feed intake (g/bird/d)	Egg to feed ratio	Qualified egg ratio (%)
30–32[1]	0	74.3	73.4	53.4	153.6	0.35	69.4
	40	76.2	77.1	58.4	154.4	0.38	73.8
	140	75.6	81.7	61.7	154.2	0.40	80.0
32–34[1]	0	75.7	84.2	65.7	156.3	0.42	83.3
	40	76.6	88.0	69.1	155.5	0.44	86.0
	140	77.1	90.4	70.3	156.4	0.45	89.5
34–36[1]	0	76.1	88.2	67.5	157.8	0.43	87.1
	40	77.3	90.9	70.9	157.6	0.45	89.6
	140	78.6	92.6	73.0	156.8	0.47	91.8
	SEM	0.39	2.65	2.00	0.53	0.01	2.67
Age[2]	30–32	75.4[b]	77.4[b]	57.8[b]	154.1[c]	0.38[b]	74.4[b]
	32–34	76.5[a]	87.5[a]	68.3[a]	156.1[b]	0.44[a]	86.3[a]
	34–36	77.3[a]	90.5[a]	70.5[a]	157.4[a]	0.45[a]	89.5[a]
	SEM	0.24	1.58	1.19	0.29	0.007	0.22
Zn[3]	0	75.4[b]	81.9[b]	62.2[b]	155.9	0.40[b]	79.9[b]
	40	76.7[a]	85.3[ab]	66.1[a]	155.8	0.42[a]	83.2[ab]
	140	77.1[a]	88.2[a]	68.3[a]	155.8	0.44[a]	87.1[a]
	SEM	0.22	1.53	1.16	0.28	0.007	1.54
	Age	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Zn	< 0.0001	0.03	0.004	0.96	0.001	0.01
	Age × Zn	0.14	0.96	0.93	0.25	0.81	0.87

[a–b] Means within a column lacking a common superscript differ ($P < 0.05$)

[1] Values represented the means of 6 replicates ($n = 6$)

[2] Values represented the means of 18 replicates ($n = 18$)

[3] Values represented the means of 18 replicates ($n = 18$)

parameters for maintaining the shelf life and the safety of eggs and egg products [7]. For poultry breeders, good eggshell quality could prevent the hatching eggs from bacterial contamination [8] to maintain hatchability performance [9]. Several studies have proved that eggshell characteristics could be affected by bird age and different housing systems in laying hens and breeder hens [10, 18]. In the current study, eggshell parameters such as weight, strength, and thickness were decreased with the increased age of duck breeders at 35–40 weeks of age regardless of rearing systems. Moreover, significant interaction between age and housing system on eggshell thickness demonstrated that no differences were observed on the average shell thickness of duck breeder eggs at 35 weeks of age under two rearing systems, whereas FC system had a lower average shell thickness than LF system in duck breeders at 40 weeks of age. It was suggested that shell thickness of a duck breeder in the FC system was decreased much greater as the age was increased. The declined eggshell quality of duck breeders in the FC system was confirmed by the comparison of eggshell quality in laying hens under the battery cage and deep litter systems [19]. It is probably due to the lack of exercise and poor bone mineral mobilization of birds in the FC system contributing to the poor mineralization during eggshell formation [4]. Another factor was that providing insufficient light intensity for laying birds at the lower tiers in multitier cage systems could induce the less calcium mobilization for eggshell formation resulting in the lower shell thickness [20]. Additionally, compared with the non-caged system, the caged system was characterized with the restricted space that allowed less movement of birds [21], which further affected physical activity, energy expenditure, and nutrient requirement of birds [6]. Therefore, it is very important to change the nutritional strategies to improve eggshell quality when duck breeders were shifted from the non-caged systems to FC systems.

Egg weight, egg production, and egg quality could be influenced by bird age [22, 23]. As the age was increased from 30 to 36 weeks old, egg production performance and yolk color of female breeders were increased linearly. The enhancement in egg production with advancing age was exhibited by the lower ovulation rate and shorter ovulation and oviposition sequence from laying early period to laying peaking period [23, 24]. Zn is a cofactor of more than 200 enzymes functioning in diverse physiological processes to maintain the

Table 4 Effects of age and dietary Zn on egg quality of duck breeders at 32, 34, and 36 weeks of age under furnished cage system

Age (weeks)	Dietary Zn (mg/kg)	Shell weight (g/egg)	Shell strength (kg/N)	Shell thickness (cm)	Haugh unit	Yolk color
32[1]	0	9.01	5.92	0.386^{ab}	72.2	8.28
	40	9.07	6.20	0.387^{ab}	73.2	8.06
	140	9.29	5.96	0.374^{bc}	76.1	8.31
34[1]	0	9.15	6.17	0.363^{c}	80.3	8.60
	40	9.01	6.08	0.360^{c}	77.6	8.67
	140	9.13	6.20	0.369^{bc}	83.1	8.32
36[1]	0	8.85	6.00	0.355^{c}	72.1	8.46
	40	8.97	5.99	0.390^{a}	71.0	8.41
	140	9.19	6.11	0.395^{a}	74.4	8.40
	SEM	0.14	0.14	0.008	1.9	0.13
Age[2]	32	9.13	6.03	0.383	73.8^{b}	8.21^{b}
	34	9.09	6.15	0.364	80.4^{a}	8.53^{a}
	36	9.00	6.03	0.380	72.5^{b}	8.42^{a}
	SEM	0.08	0.08	0.005	1.1	0.07
Zn[3]	0	9.00	6.03	0.368	74.9^{b}	8.44
	40	9.02	6.09	0.379	73.9b	8.38
	140	9.20	6.09	0.379	77.9a	8.34
	SEM	0.08	0.08	0.005	1.1	0.07
	Age	0.51	0.48	0.02	< 0.0001	0.01
	Zn	0.17	0.81	0.15	0.03	0.59
	Age × Zn	0.73	0.59	0.03	0.88	0.23

[a–b] Means within a column lacking a common superscript differ ($P < 0.05$)

[1] Values represented the means of 6 replicates ($n = 6$)

[2] Values represented the means of 18 replicates ($n = 18$)

[3] Values represented the means of 18 replicates ($n = 18$)

optimum productive performance of poultry breeders [25]. The observations that dietary Zn deficiency impaired egg production and egg quality in layer and breeder birds were well established [26]. Our results showed that dietary Zn supplementation increased egg weight, egg production, egg to feed ratio, and Haugh unit in duck breeders housed in the FC system during 30–36 weeks old. The positive effects of Zn addition on laying performance were confirmed in laying hens [27–29] and laying ducks [14]. Dietary Zn nutrition could improve the epithelium quality via increasing the protein synthesis deposition of albumen, resulting in greater values of egg production and Haugh unit. However, other studies reported that adding Zn to the diets had no effects on the characteristics of egg production of laying hens and broiler breeders [30, 31]. The differences between the studies might depend on the differences in dietary Zn levels, Zn content in basal diets, experimental periods as well as the breed differences, ages, and physiological states of birds under different rearing systems. Moreover, the diets with 140 mg Zn/kg improved laying rate and qualified egg ratio compared with the control diet, whereas no differences were observed between the control diet and diet with 40 mg Zn/kg. It is implied that dietary higher Zn level revealed the greater beneficial effect on egg production and egg quality in laying duck breeders in the FC system. Abd El-Hack et al. (2018) also stated that laying hens fed diet supplemented with 100 mg Zn/kg as Zn-Met source had greater egg production compared with birds fed basal diet supplemented with 50 mg Zn/kg as Zn-Met source [29]. One possible explanation for improvement in egg production may be the interaction of Zn with the secretion of reproductive hormones of the endocrine system during egg formation [32, 33].

Zinc is part of the CA enzyme which is essential in supplying carbonate ions during eggshell formation [34]. Numerous studies have shown that dietary Zn supplementation enhanced eggshell quality by increasing eggshell thickness and strength in laying birds [11, 12]. However, eggshell quality of laying ducks were not affected by dietary Zn level in the present study, which does not agree with results reported in laying hens [13]. The inconsistent results may be due to the differences in the Zn content and supplemental Zn levels in basal diets, experimental periods, age, and rearing systems of birds. In the present study, the basal diet containing about 29 mg Zn/kg diet seem to be sufficient for maintaining the eggshell quality in a shorter period during 30–34 weeks, according to

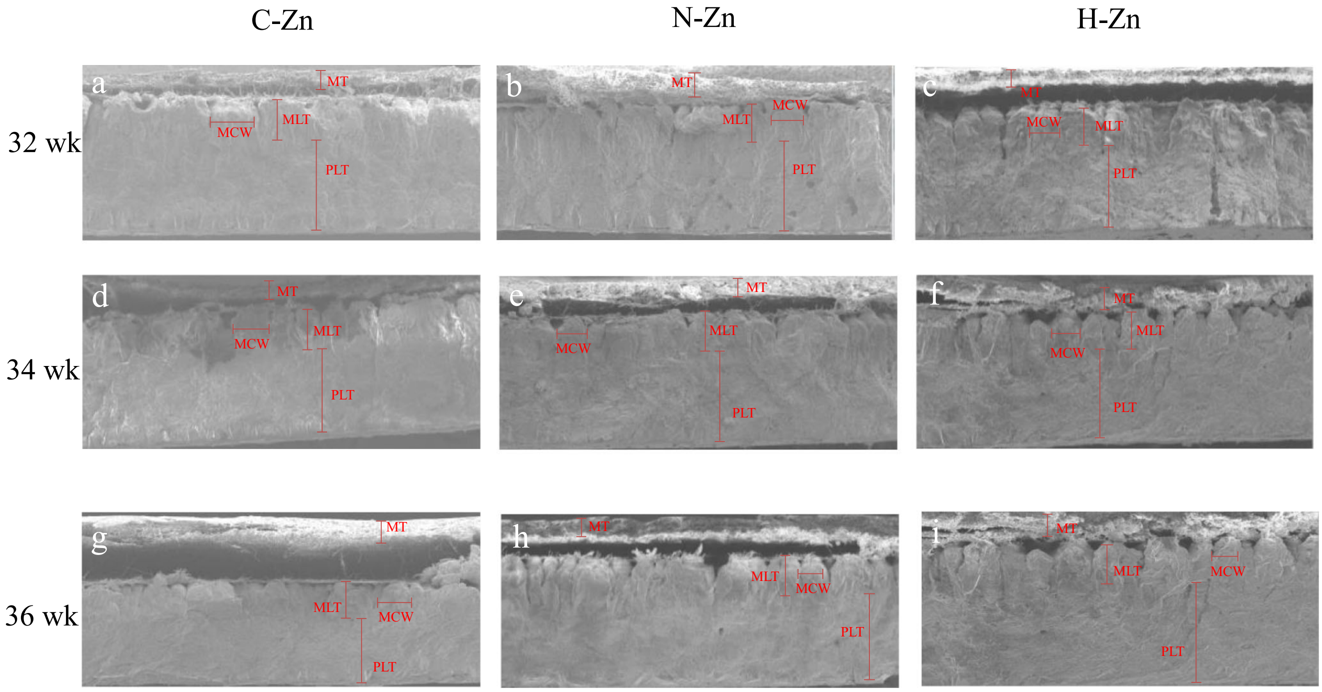

Fig. 1 Scanning electron cross-sections of eggshells from duck breeders fed C-Zn (A, D, G), N-Zn (B, E, F), and H-Zn (C, F, I) diets at 32, 34, and 36 weeks of age (×200). C-Zn, control Zn group with 0 mg Zn/kg diet; N-Zn, normal Zn group with 40 mg Zn/kg diet; H-Zn, high Zn group with 140 mg Zn/kg diet; MT, membrane thickness; MCW, mammillary cone width; MLT, mammillary layer thickness; PLT, palisade layer thickness

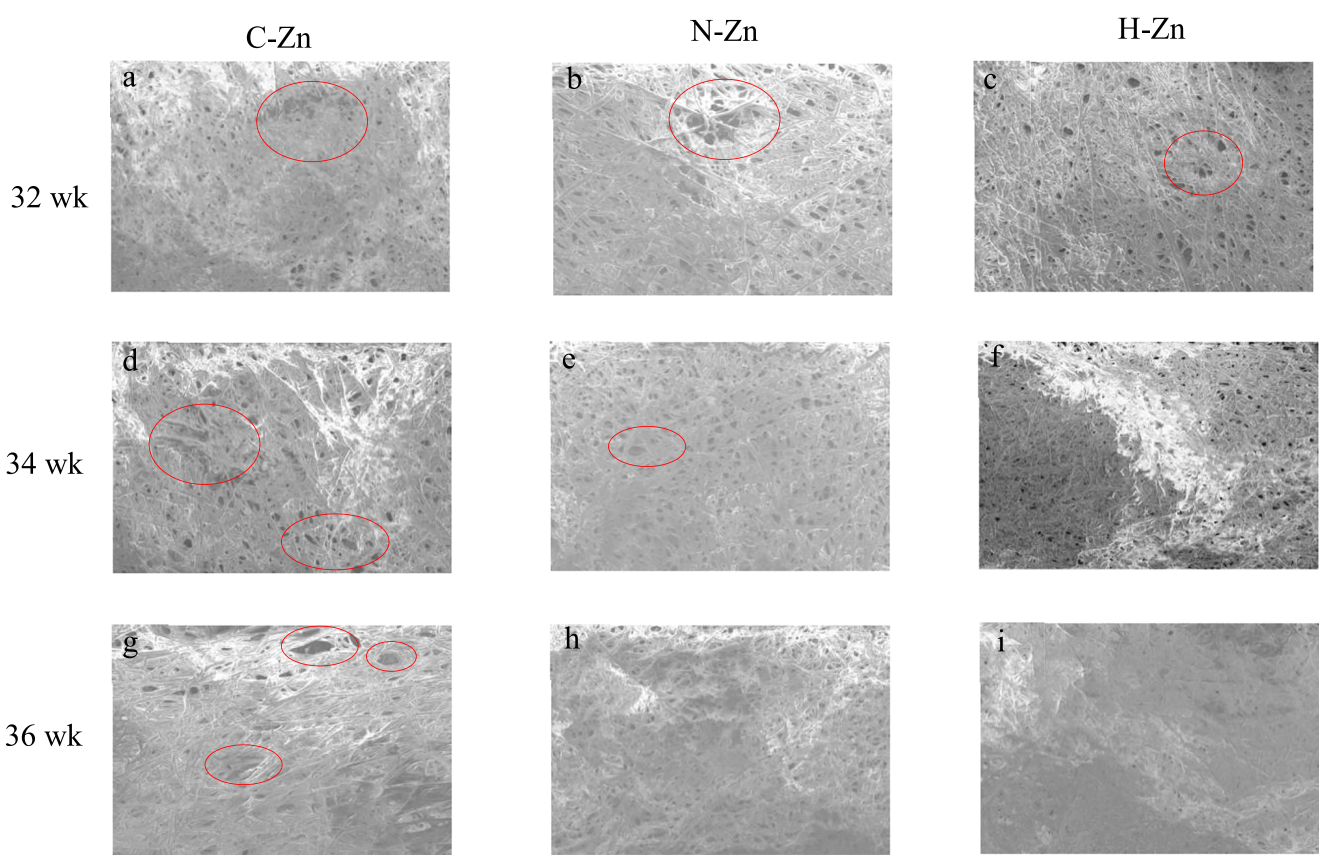

Fig. 2 Scanning electron fiber cross-linking structure of eggshell membranes from duck breeders fed C-Zn (A, D, G), N-Zn (B, E, F), and H-Zn (C, F, I) diets at 32, 34, and 36 weeks of age (×600). C-Zn, control Zn group with 0 mg Zn/kg diet; N-Zn, normal Zn group with 40 mg Zn/kg diet; H-Zn, high Zn group with 140 mg Zn/kg diet. The representative defective fiber cross-linking structure of eggshell membranes was indicated in red circle

Table 5 Effects of age and dietary Zn on eggshell microstructure of duck breeders at 32, 34, and 36 weeks of age under furnished cage system

Age (weeks)	Dietary Zn (mg/kg)	Mammillary cone width (μm)	Mammillary layer thickness (μm)	Palisade layer thickness (μm)	Membrane thickness (μm)
32[1]	0	92.8	111	276[ab]	60.7
	40	85.1	114	276[ab]	59.6
	140	79.3	102	269[ab]	62.5
34[1]	0	85.6	125	249[b]	66.2
	40	78.0	117	257[ab]	60.7
	140	74.4	124	254[ab]	51.6
36[1]	0	77.5	112	244[b]	64.9
	40	69.4	110	253[ab]	75.0
	140	61.3	106	289[a]	58.3
	SEM	5.4	6	15	6.7
Age[2]	32	85.7[a]	109[b]	274	60.9
	34	79.3[a]	122[a]	253	59.5
	36	69.4[b]	110[b]	262	66.0
	SEM	3.1	3	8	3.8
Zn[3]	0	85.3[a]	116	256	62.8
	40	77.5[ab]	114	262	67.3
	140	71.7[b]	111	271	60.4
	SEM	3.1	3	8	3.8
	Age	0.003	0.01	0.76	0.46
	Zn	0.01	0.49	0.36	0.33
	Age × Zn	0.99	0.56	0.05	0.50

[a–b] Means within a column lacking a common superscript differ ($P < 0.05$)

[1] Values represented the means of 6 replicates ($n = 6$)

[2] Values represented the means of 18 replicates ($n = 18$)

[3] Values represented the means of 18 replicates ($n = 18$)

the INRA (1989) recommendation of 20–40 mg Zn/kg of laying Muscovy duck breeders. The lacking effect of Zn addition on eggshell quality was consistent with the previous results in laying ducks fed a corn-soybean meal basal diet containing 37 mg Zn/kg during laying early and peak periods [14]. Although dietary Zn level had no effect on shell thickness of duck breeders at 32 and 34 weeks of age, whereas Zn supplementation in diets improved shell thickness of duck breeders at 36 weeks of age compared with the control diet. The interaction between age and dietary Zn on shell thickness implied that dietary Zn nutrition has a prolonged and accumulative beneficial effect on maintaining eggshell thickness of laying duck breeders. As the feeding time was increased, feeding Zn non-supplemented diet plus the lack of exercise of birds in the FC system resulted in the more severe body Zn depletion and insufficient bone Zn mobilization and then revealed the negative effect on shell thickness. Zhang et al. (2017) also indicated that Zn deficiency impaired eggshell thickness associated with the decreased CA activity during eggshell formation for the 6 weeks of experimental period [11].

The eggshell is structurally combined by organic and inorganic components [35]. The organic components of eggshell consisted of ultrastructural layers divided into shell membrane, mammillary knob, palisade, and cuticle [36]. The assembly and calcitic mineralization of different eggshell compartments are thought to be guided by organic molecules, including non-collagenous proteins and proteoglycans [37]. It has been shown that the enzymatic activity as trace cofactors influenced the formation of the mammillary layer in the region of the oviduct termed the tubular shell gland [12]. In the present study, scanning electron photographs results showed that 140 mg Zn/kg addition in diet reduced the mammillary cone width of eggshell compared with the control diet, which was in agreement with laying hens fed Mn-supplemented diet [38]. The decreased size of mammillary cones due to Zn intake implied that the number of mammillary cones per unit area on the internal shell membrane was increased. It was thought that more mammillary buttons grew into adjacent palisade column by calcitic mineralization and then resulted in a greater resistance of breakage [39]. The palisade layer accounts for about two-thirds of the thickness of the calcified eggshell. Diet supplemented with 140 mg Zn/kg increased the palisade layer thickness of duck breeders at 36 weeks of age compared with the control diet, with no effects observed in duck breeders at 32 and 34 weeks of age, which coincided exactly with the results of shell thickness in the present study. It is suggested that Zn nutrition could contribute to eggshell quality via improving the ultrastructural features. Previous study has demonstrated that proteoglycans plays a key role in the crystallization of the eggshell [40]. The Zn addition in diets could stimulate the proteoglycans synthesis to promote the eggshell nucleation and crystalline palisade growth [41, 42]. In addition, the greater Zn addition could enlarge the negative effect of the presence of high Ca concentration (3.7%) and phytate in basal hen diet due to the formation of an insoluble Ca-phytate-Zn complex [43, 44]. Zn also plays a role in deposition of albumen in the magnum and production of eggshell membranes in the isthmus [26]. Although the outer shell membrane thickness is not affected by dietary Zn levels, the poor membrane fiber formation or cross-linking structure occurred in dietary Zn deficiency and was characterized by abnormal compactness and distribution uniformity of fibers. It is implied that the enhancement of ultrastructural structure of shell membrane may produce resistant eggshells and then prevent the moisture loss of inner egg components due to Zn addition, which contributed to the qualify egg ratio and Haugh unit score in the present study.

In conclusion, dietary Zn supplementation improved the egg production performance and Haugh unit of laying duck breeders reared in the FC system during 30–36 weeks of age, displaying the greater beneficial effect for duck breeders fed a diet containing 140 mg Zn/kg. In addition, dietary high level of 140 mg Zn/kg increased ultrastructural palisade layer thickness contributing to the greater eggshell thickness of duck breeders at 36 weeks of age.

Authors' Contributions YWZ, LH, and JJS were responsible for the planning of the study, sample collections, analyses, and the manuscript writing. DQL, WCW, and YF were involved in the sample collections, biological analysis, and statistical analyses. LY and YWZ were involved in the experimental design and data interpretations. All authors read and approved the final manuscript.

Funding Information This study was sponsored by the Guangdong Provincial Natural Science Foundation for Starting Ph. D (2017A030310398 and 2018A030310202), National Natural Science Foundation of China (31802080 and 31972584), National Waterfowl Industry Program in China (CARS-42-15), National Key Research and Development Projects in China (2018YFD0501502), and Open Project Program of Key Laboratory of Feed Biotechnology, Ministry of Agriculture, Beijing, 100081, China.

Compliance with Ethical Standards

All procedures of our experiments were approved by the animal care and welfare committee institute of South China Agricultural University.

Competing Interests The authors declare that they have no competing interests.

Abbreviations *FC*, furnished cage; *LF*, littered floor; *CA*, carbonic anhydrase; *Zn*, zinc; *C-Zn*, control Zn group with 0 mg Zn/kg diet; *N-Zn*, normal Zn group with 40 mg Zn/kg diet; *H-Zn*, high Zn group with 140 mg Zn/kg diet

References

1. Xin H, Gates RS, Green AR, Mitloehner FM, Moore PA Jr, Wathes CM (2011) Environmental impacts and sustainability of egg production systems. Poult Sci 90(1):263–277
2. Sumner DA, Gow H, Hayes D, Matthews W, Norwood B, Rosen-Molina JT, Thurman W (2011) Economic and market issues on the sustainability of egg production in the United States: analysis of alternative production systems. Poult Sci 90(1):241–250
3. Lay DC Jr, Fulton RM, Hester PY, Karcher DM, Kjaer JB, Mench JA, Mullens BA, Newberry RC, Nicol CJ, O'Sullivan NP et al (2011) Hen welfare in different housing systems. Poult Sci 90(1): 278–294
4. Norgaard-Nielsen G (1990) Bone strength of laying hens kept in an alternative system, compared with hens in cages and on deep-litter. Br Poult Sci 31(1):81–89
5. Lewko L, Gornowicz E (2011) Effect of housing system on egg quality in laying hens. Ann Anim Sci 11(4):607–616
6. Karcher DM, Jones DR, Abdo Z, Zhao Y, Shepherd TA, Xin H (2015) Impact of commercial housing systems and nutrient and energy intake on laying hen performance and egg quality parameters. Poult Sci 94(3):485–501
7. Hidalgo A, Rossi M, Clerici F, Ratti S (2008) A market study on the quality characteristics of eggs from different housing systems. Food Chem 106(3):1031–1038
8. De Reu K, Messens W, Heyndrickx M, Rodenburg TB, Uyttendaele M, Herman L (2008) Bacterial contamination of table eggs and the influence of housing systems. World Poultry Sci J 64(1):5–19
9. Berrang ME, Frank JF, Buhr RJ, Bailey JS, Cox NA, Mauldin J (1998) Eggshell characteristics and penetration by Salmonella through the productive life of a broiler breeder flock. Poult Sci 77(9):1446–1450
10. van den Brand H, Parmentier HK, Kemp B (2004) Effects of housing system (outdoor vs cages) and age of laying hens on egg characteristics. Br Poult Sci 45(6):745–752
11. Zhang YN, Zhang HJ, Wang J, Yue HY, Qi XL, Wu SG, Qi GH (2017) Effect of dietary supplementation of organic or inorganic zinc on carbonic anhydrase activity in eggshell formation and quality of aged laying hens. Poult Sci 96(7):2176–2183
12. Winum JY, Innocenti A, Nasr J, Montero JL, Scozzafava A, Vullo D, Supuran CT (2005) Carbonic anhydrase inhibitors: synthesis and inhibition of cytosolic/tumor-associated carbonic anhydrase isozymes I, II, IX, and XII with N-hydroxysulfamides–a new zinc-binding function in the design of inhibitors. Bioorg Med Chem Lett 15(9):2353–2358
13. Idowu O, Ajuwon R, Oso A, Akinloye O (2011) Effects of zinc supplementation on laying performance, serum chemistry and Zn residue in tibia bone, liver, excreta and egg shell of laying hens. Int J Poult Sci 10(3):225–230
14. Chen W, Wang S, Zhang HX, Ruan D, Xia WG, Cui YY, Zheng CT, Lin YC (2017) Optimization of dietary zinc for egg production and antioxidant capacity in Chinese egg-laying ducks fed a diet based on corn-wheat bran and soybean meal. Poult Sci 96(7): 2336–2343
15. NRC (ed.): Nutrient requirements of poultry, 9th revised edition. Washington, DC.: National Academy Press; 1994.
16. Monogastriques IndlraDdléd: L'alimentation des animaux monogastriques: porc, lapin, volailles: Editions Quae; 1989.17. Al-Rawi B, Craig J, Adams A (1976) Agonistic behavior and egg production of caged layers: genetic strain and group-size effects. Poult Sci 55(2):796-807
17. Berwanger E, Vieira SL, Angel CR, Kindlein L, Mayer AN, Ebbing MA, Lopes M (2018) Copper requirements of broiler breeder hens. Poult Sci 97(8):2785–2797
18. Sokolowicz Z, Krawczyk J, Dykiel M (2018) The effect of the type of alternative housing system, genotype and age of laying hens on egg quality. Ann Anim Sci 18(2):541–555
19. Clerici F, Casiraghi E, Hidalgo A, Rossi M (2006) Evaluation of eggshell quality characteristics in relation to the housing system of laying hens. In: XII Eur Poult Conf: 2006: 10-14
20. Yildiz A, Lacin E, Hayirli A, Macit M (2006) Effects of cage location and tier level with respect to light intensity in semiconfined housing on egg production and quality during the late laying period. J Appl Poult Res 15(3):355–361
21. Duncan IJ (2001) The pros and cons of cages. World Poultry Sci J 57(4):381–390
22. Nys Y (1986) Relationships between age, shell quality and individual rate and duration of shell formation in domestic hens. Br Poult Sci 27(2):253–259
23. Johnston SA, Gous RM (2003) An improved mathematical model of the ovulatory cycle of the laying hen. Br Poult Sci 44(5):752–760
24. Johnston SA, Gous RM (2007) A mechanistic, stochastic, population model of egg production. Br Poult Sci 48(2):224–232
25. Park SY, Birkhold SG, Kubena LF, Nisbet DJ, Ricke SC (2004) Review on the role of dietary zinc in poultry nutrition, immunity, and reproduction. Biol Trace Elem Res 101(2):147–163
26. Huang L, Li X, Wang W, Yang L, Zhu Y (2019) The role of zinc in poultry breeder and hen nutrition: an update. Biol Trace Elem Res: 1–11. https://doi.org/10.1007/s12011-019-1659-0
27. Olgun O, Yildiz AO (2017) Effects of dietary supplementation of inorganic, organic or nano zinc forms on performance, eggshell

quality, and bone characteristics in laying hens. Ann Anim Sci 17(2):463–476
28. Abd El-Hack ME, Alagawany M, Amer SA, Arif M, Wahdan KMM, El-Kholy MS (2018) Effect of dietary supplementation of organic zinc on laying performance, egg quality and some biochemical parameters of laying hens. J Anim Physiol an N 102(2): E542–E549
29. Abd El-Hack ME, Alagawany M, Salah AS, Abdel-Latif MA, Farghly MFA (2018) Effects of dietary supplementation of zinc oxide and zinc methionine on layer performance, egg quality, and blood serum indices. Biol Trace Elem Res 184(2):456–462
30. Tabatabaie MM, Aliarabi H, Saki AA, Ahmadi A, Siyar SA (2007) Effect of different sources and levels of zinc on egg quality and laying hen performance. Pak J Biol Sci 10(19):3476–3478
31. Liao X, Li W, Zhu Y, Zhang L, Lu L, Lin X, Luo X (2018) Effects of environmental temperature and dietary zinc on egg production performance, egg quality and antioxidant status and expression of heat-shock proteins in tissues of broiler breeders. Br J Nutr 120(1): 3–12
32. Favier AE (1992) The role of zinc in reproduction. Hormonal mechanisms Biol Trace Elem Res 32:363–382
33. Amen MH, Al-Daraji HJ (2011) Influence of dietary supplementation with zinc on sex hormones concentrations of broiler breeder chickens. Pak J Nutr 10:1089–1093
34. Lindskog S (1997) Structure and mechanism of carbonic anhydrase. Pharmacol Ther 74(1):1–20
35. Hincke MT, Nys Y, Gautron J, Mann K, Rodriguez-Navarro AB, McKee MD (2012) The eggshell: structure, composition and mineralization. Front Biosci-Landmrk 17:1266–1280
36. Hincke MT, Nys Y, Gautron J, Mann K, Rodriguez-Navarro AB, McKee MD (2012) The eggshell: structure, composition and mineralization. Front Biosci 17(1266):80
37. Lavelin I, Meiri N, Pines M (2000) New insight in eggshell formation. Poult Sci 79(7):1014–1017
38. Xiao JF, Zhang YN, Wu SG, Zhang HJ, Yue HY, Qi GH (2014) Manganese supplementation enhances the synthesis of glycosaminoglycan in eggshell membrane: a strategy to improve eggshell quality in laying hens. Poult Sci 93(2):380–388
39. Solomon SE (2010) The eggshell: strength, structure and function. Br Poult Sci 51:52–59
40. Carrino DA, Rodriguez JP, Caplan AI (1997) Dermatan sulfate proteoglycans from the mineralized matrix of the avian eggshell. Connect Tissue Res 36(3):175–193
41. Rodriguez JP, Rosselot G (2001) Effects of zinc on cell proliferation and proteoglycan characteristics of epiphyseal chondrocytes. J Cell Biochem 82(3):501–511
42. Nys Y, Gautron J, Garcia-Ruiz JM, Hincke MT (2004) Avian eggshell mineralization: biochemical and functional characterization of matrix proteins. Cr Palevol 3(6-7):549–562
43. Linares LB, Broomhead JN, Guaiume EA, Ledoux DR, Veum TL, Raboy V (2007) Effects of low phytate barley (Hordeum vulgare L.) on zinc utilization in young broiler chicks. Poult Sci 86(2):299–308
44. Wood RJ, Zheng JJ (1997) High dietary calcium intakes reduce zinc absorption and balance in humans. Am J Clin Nutr 65(6):1803–1809