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LAYING PERFORMANCE, EGG QUALITY, AND SERUM BIOCHEMICAL INDICATORS OF LAYING DUCKS AFFECTED BY DIETARY CHITOSAN OLIGOSACCHARIDES SUPPLEMENTATION

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ABSTRACT

Chitosan oligosaccharides (COs) have varying physiologic activities. This work aims to explore effects of dietary COs on the laying performance, egg quality, and plasma biochemical indicators of laying ducks. Four diets were formulated by supplementing different dosages (0, 25, 50, and 100 mg/kg) of COs in basal diet. A total of 288 Suyou No.2 healthy laying ducks at peak egg production stage with age of 28 weeks, similar weights (1500 ± 106 g) and egg laying rates were randomly evenly assigned to four groups, with 6 replicates in each group. Dietary 50 mg/kg of COs increased daily egg production (EP), egg mass (EM), egg weight (EW), eggshell strength, yolk color, serum superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities, and immunoglobulin (Ig) G, Ig A, Ig M, and high-density lipoprotein levels (HDL-C). Conversely, it decreased the feed conversion ratio (FCR), yolk percentage, and serum malondialdehyde (MDA), total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) levels of laying ducks. Nevertheless, a high dosage of COs (100 mg/kg) neither decreased nor improved the efficiency. The optimum dose of COs supplementation required for the maximum egg mass of laying ducks was 61.41 mg/kg. These results indicated that COs supplementation at an appropriate dosage could ameliorate the laying performance, egg quality, and health condition of laying ducks.

Keywords: dietary, chitosan oligosaccharides, laying ducks.

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INTRODUCTION

Egg duck breeding is part of the traditional animal husbandry in China, which also has the tradition of processing century eggs and salted eggs (Cao *et al.,* 2022). China's egg duck inventory has reached approximately 300 million in the past decade; its annual production of duck eggs can reach 6 million tons (Chen *et al.,* 2021). Good laying performance and egg quality are important for egg duck breeding industry and depend on the health status of laying ducks. Many substances, such as cassava starch-extraction-residue meal (Abouelezz *et al.,* 2022), probiotics (Cao *et al.,* 2022), chromium propionate (Chen *et al.,* 2021), corn bran (Hou *et al.,* 2023), 2-hydroxy-4-methyl(thio)butanoic acid (Zhang *et al.,* 2023a), tryptophan (Zhang *et al.,* 2021), and manganese (Zhang *et al.,* 2020), have been used to ameliorate the laying performance, egg quality, and health condition of laying ducks.

Chitin is a biopolymer formed by acetylglucosamine. This polysaccharide is distributed in the cell wall of yeast, as well as in the exoskeletons of insects and arthropods. Chitosan is a mixture of most acetylglucosamine residues and a small amount of glucosamine residues obtained through deacetylation reaction of chitin. Chitosan oligosaccharides (COs) are oligosaccharides derived from the hydrolysates of chitosan. COs have varying physiological effects, such as antioxidant (Liu *et al.,* 2023), antimicrobial (Zhang *et al.,* 2024a), antiviral (Lan *et al.,* 2023), immunomodulatory (Xiong *et al.,* 2009), and hypolipidemic activities (Wang *et al.,* 2017a). COs have recently been used as a dietary additive to ameliorate the growth performance of crayfish (*Cherax quadricarinatus*) (Zhang *et al.,* 2024b), and sea cucumber (*Apostichopus japonicas*) (Wang *et al.,* 2017b).

Whether COs dietary supplementation improves the laying performance, egg quality, and health condition of laying ducks is worthy of further study. Hence, this study aims to explore the influences of COs dietary supplementation on the laying performance, egg quality, and serum biochemical parameters of laying ducks.

MATERIALS AND METHODS

Preparation of diet: Food-grade COs with a purity > 98% was obtained from Pulan Biotechnology Co., Ltd., Hangzhou, China. Different dosages (0, 25, 50, and 100 mg/kg) of COs were suplemented to basal diet to formulate four test diets (Table 1). The feeds were prepared using a duck-feed extruder (SZLH 420, Henan Qiangfu Machinery Co., Ltd., Wuzhi, Henan, China).

Birds and design: This study was performed using a completely randomized design. The experiment was performed at Lianyungang Livestock and Poultry Farm from 10 March 2023 to 14 April 2023. A total of 288 Suyou No.2 healthy laying ducks at peak egg production stage with age of 28 weeks, similar weights (1500 ± 106 g) and egg laying rates were randomly evenly assigned to four groups, with 6 replicates in each group. The laying ducks were fed with 0 , 25 , 50, and 100 mg/kg COs. The pretrial period was 7 days, and the main trial period was 28 days.

Single-row semi-open type duck houses were selected for the experiment and cleaned and disinfected before use. Ground-level farming was adopted to raise the laying ducks, which were allowed to freely feed and drink water. Artificial lighting was provided from 18:00 to 06:00 the next day, and natural lighting was provided for the rest of the day. The eggs were picked up, and the duck coops were cleaned every day at 08:00 and 18:00.

Laying performance: During the experiment, the total egg production, number of crushed or soft eggs, EW, and feed consumption of laying ducks in each group were recorded daily during the second to fifth week period. After the experiment, the feed intake (FI), FCR, daily egg production weight, egg production rate, and soft egg breaking rate of each group of laying ducks were calculated.

Egg quality: Six eggs were randomly collected from each group in the 2nd and 4th weeks. A PD-151 type vernier caliper was used to measure the short and long diameters of eggs, and the egg shape index was calculated. An eggshell strength tester (MODEL-2, Shimpo, Japan) was used to measure eggshell strength. A spiral micrometer was employed to measure the thickness of the egg shape indices, and the average thickness of eggshells were calculated. An egg quality tester (EMT-2500, robotmation, Japan) was used to measure protein height, Haugh unit, and egg yolk color. An analytical balance (ME-T, Mettler Toledo) was used to determine the weight of eggs and yolks, and the weight of egg white, relative eggshell weight, relative egg protein weight, and relative weight of egg yolk were calculated.

Serum biochemical indicator: Six laying ducks were randomly selected from each group after the feeding experiment. Blood was collected from the pterygoid vein, placed in an ethylenediaminetetraacetic acid anticoagulant tube, and centrifuged at 3,000 × *g* for 15 min. The plasma was collected for detecting plasma biochemical indicators. GPX activity, SOD activity, IgG, IgA, and IgM levels were assayed using ELISA kits (Yuanxin Biotechnology Co., Ltd., Shanghai, China). Serum MDA, TC, LDL-C, TG, and HDL-C levels were determined using commercial assay kits (Yuanxin Biotechnology Co., Ltd., Shanghai, China).

Statistical analysis: The data were expressed as the mean ± SD (n = 6). ANOVA One-way with a completely randomized design was used to analyze experimental data. Duncan's multiple-range test was used to determine significant differences, and $p \leq 0.05$ indicated significant differences.

RESULTS

Laying performance: Table 2 shows the lack of significant differences in daily egg production, EW, FI, and FCR among the 0, 25, and 100 mg/kg COs groups. Dietary 50 mg/kg of COs improved daily egg production, EW, and EM and reduced the FCR ($P \leq 0.05$). The optimum supplementary COs dose required for the maximal egg production of laying ducks is 61.41 mg/kg (Fig. 1).

Egg quality: Table 3 shows the lack of significant differences in egg eggshell strength, shape index, yolk percentage, eggshell thickness, albumen height, albumen percentage, Haugh unit, and yolk color among the 0, 25, and 100 mg/kg COs groups. Meanwhile, dietary COs supplementation at a dosage of 50 mg/kg increased eggshell strength, yolk percentage and yolk color and decreased yolk TG compared with those of 0 mg/kg COs group ($P \leq 0.05$).

Serum biochemical indicator: Table 4 shows the lack of significant differences in all serum indicators between the 50 and 100 mg/kg COs groups. However, dietary COs supplementation at a dosage of 50 mg/kg increased egg shell strength, serum SOD activity, GPX activity, and IgG, IgA, IgM, and HDL-C levels and decreased serum TC, TG, and LDL-C levels ($P \leq 0.05$).

Table 1. Composition of the basal diet of laying duck (%).

Ingredient	Content (%)	Nutrition (%)	Content
Corn	55	AME (MJ/kg)	10.66
Soybean meal	27	Protein	18.07
Limestone	8.8	Fiber	3.11
Wheat bran	6.5	Lys	0.85
$CaHO_4$	1.3	Met	0.45
NaCl	0.2 Cys	0.30	
Met	0.18 Ca	3.62	
Lys	0.02	P	0.61
Premix^{a}	0.20		

^{a}Supplements to 1 kg of basal diet: VA 9,000 IU, VD_3 2000 IU, VE 40 IU, Choline chloride 600 mg, Nicotinic acid 30mg, Pantothenic acid 11 mg, VB_2 8.0 mg, Pyridoxine 2.5 mg, VB_1 2.0 mg, VK 0.8 mg, Folic acid 0.5 mg, Biotin 0.2 mg, Fe ($FeSO_4$) 80 mg, Zn (Zn SO_4) 60 mg, I [$Ca(IO_3)_2$] 0.3 mg, Se (Na_2SeO_3) 0.5 mg, Mn (Mn SO_4) 80 mg.

Table 2. Effect of dietary COs on the productive performance of laying ducks. EW, egg weight; EM (g), egg mass = Day egg production (%) × EW (g)/100; FI, Feed intake; FCR, feed conversion ratio = FI (g) / EM (g)

Item	Diets (mg/kg)			
	0 (Control)	25	50	100
Day egg production (%)	72.87 ± 1.06^{a}	74.91 ± 1.27^{ab}	81.73 ± 1.39^{c}	75.83 ± 1.15^{b}
EW (g)	61.24 ± 0.42^{a}	63.28 ± 0.44^{ab}	65.15 ± 0.47^{b}	63.73 ± 0.41^{ab}
EM (g)	44.63 ± 0.62^{a}	47.40 ± 0.72^{b}	53.25 ± 0.86^{c}	48.33 ± 0.68^{b}
FI (g/hen/d)	132.18 ± 3.25	133.04 ± 3.31	134.29 ± 3.41	133.07 ± 3.28
FCR (g/g)	2.96 ± 0.09^{b}	2.81 ± 0.08^{ab}	2.52 ± 0.05^{a}	2.75 ± 0.07^{ab}

^{abc}Means with different superscripts in a row differ significantly (P≤0.05). Values are the mean ± SD (n = 6).

Table 3. Effects of dietary COs on egg quality of laying ducks.

Item	Diets (mg/kg)			
	0 (Control)	25	50	100
Egg shape indices	1.35 ± 0.05	1.36 ± 0.06	1.37 ± 0.08	1.36 ± 0.04
Egg shell strength (kg/cm^2)	42.69 ± 7.03^{a}	45.16 ± 8.13^{ab}	48.37 ± 8.81^{b}	45.28 ± 8.15^{ab}
Egg shell thickness (mm)	0.24 ± 0.02	0.23 ± 0.02	0.24 ± 0.02	0.23 ± 0.02
Yolk percentage (%)	33.47 ± 0.38^{c}	32.16 ± 0.35^{bc}	30.48 ± 0.32^{ab}	29.37 ± 0.31^{a}
Albumen percentage (%)	27.51 ± 0.44	27.52 ± 0.38	27.61 ± 0.05	27.75 ± 0.07
Albumen height (mm)	6.28 ± 0.75	6.33 ± 0.81	6.42± 0.93	6.47 ± 0.77
Yolk colour	9.17 ± 1.22^{a}	10.13 ± 1.27^{ab}	11.31 ± 1.28^{b}	12.21 ± 1.21^{bc}
Haugh unit	75.86 ± 5.13	76.92 ± 5.46	77.72 ± 5.53	77.81 ± 5.42
Yolk TG (mmol/g protein)	0.53 ± 0.02^{c}	0.49 ± 0.02^{bc}	0.45 ± 0.02^{ab}	0.41 ± 0.02^{a}

^{abc}Means with different superscripts in a row differ significantly (P≤0.05). Values are the mean ± SD (n = 6).

Table 4. Effects of dietary COs on serum biochemical indicators of laying ducks.

Item	Diets (mg/kg)			
	0 (Control)	25	50	100
SOD (U/mL)	80.07 ± 6.42^{a}	82.31 ± 6.58^{ab}	85.48 ± 7.03^{b}	85.64 ± 7.04^{b}
GPX (U/mL)	135.37 ± 9.26^{a}	138.83 ±10.35^{ab}	148.59 ± 11.75^{c}	151.37 ± 11.86^{c}
MDA (μmol/mL)	10.48 ± 0.87^{bc}	10.03 ± 0.84^{b}	9.57 ± 0.82^{ab}	8.26 ± 0.71^{a}
IgG (g/L)	1.79 ± 0.3^{a}	1.94 ± 0.35^{ab}	2.34 ± 0.41^{b}	1.95 ± 0.34^{ab}
IgA (g/L)	0.21 ± 0.0^{a}	0.23 ± 0.02^{ab}	0.25 ± 0.03^{b}	0.23 ± 0.01^{ab}
IgM (g/L)	0.34 ± 0.02^{a}	0.45 ± 0.03^{ab}	0.57 ± 0.04^{b}	0.46 ± 0.02^{ab}
TG (mmol/L)	4.11 ± 0.54^{bc}	3.41 ± 0.43^{b}	2.61 ± 0.32^{ab}	2.53 ± 0.31^{a}
TC (mmol/L)	4.03 ± 1.03^{bc}	3.26 ± 0.9^{b}	2.71 ± 0.79^{ab}	2.56 ± 0.76^{a}
LDL-C (mmol/L)	5.94 ± 0.8^{bc}	5.17 ± 0.75^{b}	4.09 ± 0.66^{ab}	3.59 ± 0.56^{a}
HDL-C (mmol/L)	3.17 ± 0.31^{a}	3.51 ± 0.42^{ab}	4.47 ± 0.61^{b}	4.81 ± 0.72^{bc}

^{abc}Means with different superscripts in a row differ significantly (P≤0.05). Values are the mean ± SD (n = 6).

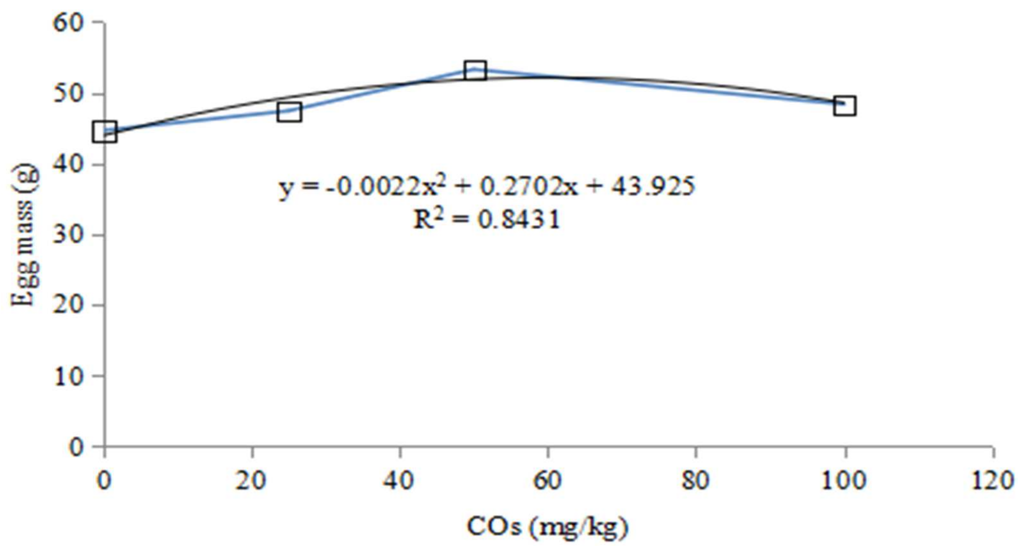

Fig. 1. Quadratic polynomial regression equation of egg mass against dose of chitosan oligosaccharides (COs).

DISCUSSION

The laying performance is affected by many factors, such as heredity, age, health status, nutritional status, and husbandry management (Hou *et al.,* 2023). Dietary 50 mg/kg of COs increased the daily EP, EW, and EM and decreased the FCR compared with control group. COs have antiviral (Lan *et al.,* 2023) and immunomodulatory activities (Xiong *et al.,* 2009) and thus can decrease virus infection and enhance the immunity of laying ducks, resulting in improved laying performance. In addition, the biosynthesis of protein and lipid requires a reduction environment. COs have antioxidant activity (Liu *et al.,* 2023) and can help maintain a reduction environment and improve the laying performance of laying ducks. However, a high dose of COs (100 mg/kg) decreased the efficiency compared with a moderate dose (50 mg/kg); this could be because of the hypolipidemic activity of COs (Wang *et al.,* 2017).

Egg quality is one of important indicators for evaluation of the egg-production efficiency of commercial laying ducks. COs have antiviral antibacterial (Zhang *et al.,* 2024a), antiviral (Lan *et al.,* 2023), and immunomodulatory activities (Xiong *et al.,* 2009) and can improve the health status of the digestive tract of laying ducks, thus promoting the absorption of mineral elements and improving egg shell strength. With the improvement of living standards, people increasingly pay attention to dietary health, such as high-protein and low-fat foods. In this work, dietary COs supplementation at appropriate dose decreased the yolk percentage and yolk TG; this could be because of the hypolipidemic activity of COs (Wang *et al.,* 2017a). A dark yolk color has a positive impact on egg quality (Zhang *et al.,* 2023a). Dietary supplementation of appropriate dose of COs darkened the yolk color compared with the control group. COs have antioxidant activity (Liu *et al.,* 2023) and may inhibit the oxidation of carotenoids in egg yolks, thereby intensifying the yolk color (Zhang *et al.,* 2023a).

Serum biochemical indicators are also important indicators for disease diagnosis and detection, and their changes can reflect the health status of animals (Zhang *et al.,* 2021). The metabolism of organisms requires energy, and the production of energy requires oxidative respiration that produces ROS. ROS accumulation disrupts the balance between oxidation and antioxidant in organisms (Cheng, 2019). Excessive ROS damages biomolecules, produces MDA, and leads to cell apoptosis and tissue damage (Pan *et al.,* 2018). SOD, GPx, and other antioxidants can maintain the balance between oxidation and antioxidant in organisms and protect the biological organs from oxidative damage (Zhang, 2018). Serum IgG, IgA, and IgM levels reflect the immunity status of animals (Cheng and Wu, 2019), and serum lipid levels reflect the metabolism ability of lipid of animals (Zhang *et al.,* 2023b). Dietary COs supplementation at appropriate dose improved serum SOD and GPx activities and reduced serum MDA level, which could be due to the antioxidant activity of COs (Wang *et al.,* 2017). It also increased serum IgG, IgA, and IgM levels because of the immunomodulatory activity of COs (Xiong *et al.,* 2009). The reduced serum TC, TG, and LDL-C levels and improved serum HDL-C level of laying ducks are because of the hypolipidemic activity of COs (Wang *et al.,* 2017). Therefore, dietary supplementation of appropriate dose of COs improves the health status of laying ducks.

In conclusion, dietary COs supplementation at 61.41 mg/kg in laying ducks was considered as an appropriate dose for improved the laying performance, egg quality, and health status.

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