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Effects of water supplemented with *Bacillus subtilis* and photosynthetic bacteria on egg production, egg quality, serum immunoglobulins and digestive enzyme activity of ducks

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ABSTRACT

We investigated the effects of water supplemented with *Bacillus subtilis* and photosynthetic bacteria (PSB) on egg production, egg quality, serum immunoglobulins and digestive enzyme activities of Jinyun ducks. A total of 400 healthy Jinyun ducks (all aged 58 weeks) were randomly allotted to 5 treatments, with 4 replicates per treatment and 20 ducks in each replicate. The ducks were fed a standard diet for 15 days and the experimental diet for 54 days. Experimental design is as follows: negative-control group C1 was given no supplementation, control group C2 was given pool water (PW) with added PSB but not *B. subtilis*. Test groups T1, T2 and T3 were given the same amount of PSB in the PW and varying levels of *B. subtilis* in drinking water. Results suggest that PSB combined with *B. subtilis* had beneficial effects on the laying rate, feed-to-egg ratio, serum IgG, egg quality, especially in albumen height and Haugh unit. Additionally, PSB alone can increase the yolk-to-egg ratio, albumen height and Haugh unit.

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Photosynthetic bacteria;
B. subtilis; egg production;
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ABBREVIATIONS

PSB: Photosynthetic bacteria;
PW: pool water; DW: drinking
water

1. Introduction

Photosynthetic bacteria (PSB) are the oldest prokaryotes in the world, which are widely distributed; it can use organic acids, alcohols and other organic materials as the electron donor and carbon source for the photosynthesis. Furthermore, it can also tolerate water with high concentrations of organic-substance contamination, and can decompose the organic contaminants in water (Zhou et al. 2014; Mekjinda & Ritchie 2015).

Bacillus subtilis is a non-pathogenic gram-positive bacterium, it can secrete varieties of digestive enzymes (such as protease, amylase, lipase) and these enzymes enhanced digestion and nutrients' absorption (Sonenshein et al. 2000; Kodama et al. 2007; Liu et al. 2014). It is well known for its positive effects, such as enhancing immune function of the body (Lee et al. 2014), promoting growth (Hooge et al. 2004), increasing egg-laying rate (Xu et al. 2006; Ribeiro et al. 2014), improving egg quality (Mohan et al. 1995; Abdelqader et al. 2013) and adjusting microflora balance of intestinal (Lee et al. 2014):

Recently, with the improvements in animal husbandry, the levels of organic matter in water is increasing, especially in duck breeding. Lowering and purifying water pollution at the same time during the animal rearing is the most simple, direct and valid method. Studies have confirmed that PSB-

alone treatment can purify water pollution, but rarely paper focused on poultry nutrition. In this study, we investigated the combination effects of PSB alone or together with *B. subtilis* on egg production and egg quality of Jinyun ducks, especially focused on feed conversion ratio and digestive enzyme activity in digestive tract. Further research is necessary to study the effects of PSB in pool water on duck breeding in the livestock farm.

2. Materials and methods

2.1. Test materials and test facilities

2.1.1. Materials

Four-hundred, healthy, 58-week-old Jinyun ducks (the average laying rate is 74%) were procured from Zhejiang Wulian Farming Limited Company. *B. subtilis* (2.55×10^9 viable spores g^{-1}) and PSB (*Rhodospseudomonas palustris*, 6.7×10^8 cfu mL^{-1}) were obtained from the Institute of Plant Protection and Microbiology, Zhejiang Academy of Agriculture Science.

Calipers (mm), Digital micrometer (karl deut sch MODEL-1061), Egg shell force gauge (MODEL-T1), Egg multi tester (EMT-5200) and Emperor balance (Mettler Toledo pL203-IC) were provided by Institute of Animal Husbandry and Veterinary

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Science, Zhejiang Academy of Agricultural Science. Ultraviolet-visible spectrophotometer 210 Plus and Automatic Soxhahl Solvant Extractor (FOSS2055) were provided by Zhejiang University, China.

2.1.2 Test facilities

Duck pens consisted of an indoor egg area, outdoor stadium and an outdoor 2.5 m × 1.8 m × 0.25 m concrete pool. Drinking water was supplied via a 3.75 L drinker.

2.2. Experimental design and feeding management

2.2.1. Experimental design

The 400 Jinyun ducks were randomly divided into 5 treatment groups, with 4 replicates per treatment and 20 ducks in each replicate: negative-control group C1, no water supplements; control group C2, PW supplemented with 20 mg L⁻¹ PSB; group T1, 20 mg L⁻¹ PSB added to PW and 125 mg L⁻¹ *B. subtilis* added to DW; group T2, 20 mg L⁻¹ PSB added PW and 250 mg L⁻¹ *B. subtilis* added to DW; and group T3, 20 mg L⁻¹ PSB added to PW and 375 mg L⁻¹ *B. subtilis* added to DW.

The animal care and use protocol was approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University and performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals (China, 1988) and the Standards for the administration of experimental practices (Jiangsu, China, 2008).

2.2.2. Feeding management

Studies were conducted from 1 August 2014 to 10 October 2014, and included 15 days for prefed and 54 days for the fed. Food and water were provided twice a day, at 0800 and 1500 respectively. The composition and nutrient analysis of the basal diet are shown in Table 1.

To ensure the ducks could feed ad libitum throughout the experimental phase, the amount of feed provided was adjusted daily according to the amount of feed intake during the prefed period and the previous day's feed intake during the fed period. The pens were lit 24 h a day, with natural light during the day and artificial light from 1700 to 0800. All the 400 ducks survived till the end of the experimental period.

Table 1. Composition and main characteristics of the basal diet.

Ingredients	Content (g kg ⁻¹)	Nutrient	Content (g kg ⁻¹)
Maize grain	400	Metabolizable energy	11.2 ^b
Wheat	290	Crude protein	16.5
Soybean meal	120	Total phosphorus	0.70
Wheat bran	90	Total calcium	3.35
Calcium hydrophosphate	12	Total lysine	0.79
Stone powder	80		
Salt	3	Total methionine	0.40
Premix ^a	5	Ether extract	29.0

^aSupplied per kilogram of diet: vitamin A 1500 U, cholecalciferol 200 U, vitamin E (DL- α -tocopherylacetate) 10 U, riboflavin 3.5 mg, pantothenic acid 10 mg, niacin 30 mg, cobalamin 10 μ g, choline chloride 1000 mg, biotin 0.15 mg, folic acid 0.5 mg, thiamine 1.5 mg, pyridoxine 3.0 mg, Fe 80 mg, Zn 40 mg, Mn 60 mg, I 0.18 mg, Cu 8 mg, Se 0.3 mg.

^bUnit: mJ kg⁻¹.

PSB was added once a week to the PW, and the amount added was calculated based on the size of the cement pool and the desired concentration. The amount of *B. subtilis* was added to DW twice a day, and the amount was calculated from the specifications of the drinker and the desired concentration.

2.3. Sampling and handling

Once the experiment was complete, all ducks were fasted for 12 h (water was still provided). Blood was collected in anticoagulant tubes by jugular vein puncture from two randomly selected ducks in each replicates using a disposable vacutainer tube without anticoagulants. After concentrations (3000 g for 15 min at 4°C), serum samples were separated and stored at -20°C and later analysed for concentrations of immunoglobulins (IgG, IgA and IgM). The contents of the duodenum were collected and stored at -80°C and later analysed for digestive enzyme activity. Six eggs, each near the average weight, were randomly selected from each replicate and later analysed for egg quality (eggshell thickness, egg shape index, eggshell strength, albumen height, Haugh unit and relative yolk to egg ratio); this was completed within 12 h of sampling. The other three eggs were randomly selected at the end of this study and later analysed for the content of cholesterol and crude fat in eggs.

2.4. Measurement methods

2.4.1. Egg production performance

Daily feed provided, actual feed intake, number of eggs laid and individual egg weight were recorded. Average daily feed intake, average egg weight and feed-to-egg ratio were calculated at the end of the experiment.

2.4.2. Egg quality

The egg shape index was measured (in mm) with a caliper and the aspect ratio was calculated to determine the shape index. Eggshell thickness was measured with a digital micrometer (karl deut sch MODEL-1061); measurements at the blunt and sharp ends and the middle of egg were averaged to determine the overall eggshell thickness. Eggshell strength was measured using an eggshell force gauge (MODEL-T1). Albumen height, yolk colour and Haugh units were measured with an automatic egg analyser (Egg Multi Tester, EMT-5200). Egg weight and yolk weight were weighted with an electronic balance (Mettler Toledo pL203-IC).

2.4.3. Cholesterol and crude fat content in eggs

Three eggs from each replicate were randomly selected and later analysed for the content of cholesterol and crude fat in eggs. An Ultraviolet-visible spectrophotometer 210 Plus was used to measure cholesterol content, and an Automatic Soxhahl Solvant Extractor (FOSS2055) was used to measure the crude fat content.

2.4.4. Serum immunoglobulins

Serum IgA, IgM and IgG content were detected with a Duck Immunoglobulin A (IgA) ELISA kit, Duck Immunoglobulin M

(IgM) ELISA kit and a Duck Immunoglobulin G (IgG) ELISA kit, respectively, following the manufacturer's instructions. The kits were purchased from Nanjing Jiancheng Bioengineering Institute.

2.4.5. Digestive enzyme activity

Duodenum contents were collected in sterilized microtubes and stored at -70°C until analysis. The samples were homogenized with a phosphate buffer (at a ratio of 1:9) in an ice water bath, centrifuged at 2500 g for 10 min, and then the supernatant was collected and diluted for digestive enzyme activity analysis. Trypsin, Lipase and amylase activity was detected with a Trypsin assay kit, Lipase assay kit and α -Amylase Assay Kit, respectively, following the manufacturer's instruction. The kits were purchased from Nanjing Jiancheng Bioengineering Institute.

3. Statistical analysis

Data generated in the present study were subjected to statistical analysis using one-way ANOVA of SPSS17.0 in a randomized complete block design. All data were reported as the mean \pm SEM. When significant differences were identified among treatments, Duncan's test was used for multiple comparisons. The replicate was used as the experimental unit for the analysis of egg production, egg quality, and the cholesterol and crude fat content in eggs. P -values $< .05$ were considered significant.

4. Results and discussion

4.1. Egg production performance

There was no significant difference in the average daily feed intake among the treatments ($P > .05$) (Table 2).

Compared to negative-control group C1, the feed-to-egg ratio decreased by 8.0% ($P < .05$) in group T1, and by 9.00% ($P < .05$) in group T3. The laying rate improved by 6.24% ($P < .05$) in group T3. These groups had supplementation with PSB in PW alone or in combination with *B. subtilis* in DW (Table 2).

Moreover, when compared to group C2, the average egg weight decreased by 1.76% ($P < .05$) in group T2, the laying rate improved by 5.77% ($P < .01$), 4.05% ($P < .05$) and 8.86% ($P < .01$) in group T1, T2 and T3, respectively. These groups all had supplementation with PSB in PW and varying levels of *B. subtilis* in DW (Table 2).

It is well known that no life can live long without water because water is needed for metabolism in biological body. In this study, the results suggested that the laying rate improved,

feed-to-egg ratio decreased when both PSB and *B. subtilis* were used. Few articles have studied the effects of *B. subtilis* on laying rate of laying hens, but their results were discordant. Xu et al. (2006) studied the effects of basal diet supplemented with 500 mg kg^{-1} *B. subtilis* on laying rate of 21–42 week-old laying hens. Their studies showed that *B. subtilis* supplementation can significantly increase the laying rate. Mahdavi et al. (2005) investigated the effects of probiotics (*B. subtilis* and *Bacillus licheniformis*) on laying rate. However, their findings showed that probiotics' supplementation had little effect on laying rate, and high dose of *B. subtilis* can reduce the production performance because probiotics can destroy the digestion systems and prevent the nutrients from getting absorbed.

Results also showed that the laying rate tended to increase and the feed-to-egg ratio tended to decrease when the DW supplemented with the increasing dose of *B. subtilis*. The maximum egg production performance was observed in test group T3. Nahashon et al. (1994) studied the effects of *B. subtilis* on laying hens. Their studies showed that *B. subtilis* can improve digestion and absorption of calcium and phosphorus.

PSB alone or combined with *B. subtilis* treatment had little effects on the daily feed-intake. Abdelqader et al. (2013) investigated the effects of basal diet supplemented with *B. subtilis* on average feed intake of post-laying hens. Their findings suggested that *B. subtilis* can increase the average feed intake. Their findings were not consistent with this study. PSB alone treatment significantly increased the average egg weight; however, no other article has reported this before. It means that PSB had some beneficial effects on average egg weight.

Overall, it appears that treatment with PSB alone or combined with *B. subtilis* has some beneficial effects on duck egg production, and the effect of the combination on egg production was greater than PSB alone.

4.2. Egg quality

Eggshell thickness, shape index, shell strength, average egg weight, yolk colour values and yolk weight of the eggs showed no significant difference among the treatments ($P > .05$) (Table 3).

Compared to group C1, the yolk to egg weight increased by 4.73% ($P < .05$) in group C2. The albumen height improved by 18.79% ($P < .01$) in group C2 and by 19.08% ($P < .01$) in group T1. The Haugh unit improved by 10.30% ($P < .01$) in group C2 and 10.06% ($P < .01$) in group T1 (Table 3).

In addition, when compared with group C2, the albumen height decreased by 11.19% ($P < .05$) in group T3, the Haugh unit decreased by 6.4% ($P > .05$) in group T3 (Table 3).

Table 2. Effects of supplementing the water offered Jinyun ducks with *B. subtilis* and PSB on the production performance.

Items	Groups				
	C1	C2	T1	T2	T3
Average daily feed intake (g)	170.87 \pm 18.87	171.64 \pm 18.06	171.19 \pm 21.57	169.33 \pm 18.31	171.96 \pm 15.33
Average egg weight (g)	70.77 \pm 3.24ab	71.72 \pm 3.09b	71.10 \pm 2.99ab	70.46 \pm 6.34a	71.03 \pm 2.31ab
Feed-to-egg ratio (g g ⁻¹)	3.26 \pm 1.19b	3.20 \pm 0.52ab	3.00 \pm 0.46a	3.19 \pm 1.63ab	2.97 \pm 0.44a
Laying rate (%)	77.93 \pm 14.73abAB	76.05 \pm 9.95aA	80.44 \pm 9.92bcBC	79.13 \pm 8.27bAB	82.79 \pm 11.04cBC

Notes: Values are expressed as mean \pm SEM for four biological replicates. Values with different lowercased and uppercased letters in the same row differ significantly ($P < .05$ and $P < .01$, respectively).

Table 3. Effects of supplementing the water offered Jinyun ducks with *B. subtilis* and PSB on egg quality.

Items	Groups				
	C1	C2	T1	T2	T3
Eggshell thickness (mm)	0.42 ± 0.04	0.41 ± 0.04	0.41 ± 0.03	0.41 ± 0.03	0.42 ± 0.03
Egg shape index	1.38 ± 0.05	1.37 ± 0.07	1.36 ± 0.05	1.37 ± 0.06	1.39 ± 0.06
Eggshell strength (kg cm ⁻²)	4.31 ± 0.63	4.24 ± 0.57	4.11 ± 0.81	3.96 ± 0.71	3.87 ± 0.92
Average egg weight (g)	77.16 ± 3.24	74.39 ± 3.04	75.94 ± 4.25	74.41 ± 4.66	74.67 ± 4.84
Albumen height (mm)	6.92 ± 1.01aA	8.22 ± 1.59bB	8.24 ± 1.34bB	7.48 ± 1.09abAB	7.30 ± 1.32aAB
Yolk colour score	13.51 ± 0.61	13.65 ± 0.86	13.40 ± 0.60	13.21 ± 0.33	13.58 ± 0.79
Haugh unit	79.65 ± 6.69aA	87.85 ± 9.36bB	87.66 ± 8.00bB	83.86 ± 6.94abAB	82.23 ± 8.78abAB
Average yolk weight (g)	24.44 ± 0.91	24.70 ± 2.11	24.60 ± 1.97	23.85 ± 1.79	24.42 ± 1.70
Yolk-to-egg ratio (%)	31.70 ± 1.28a	33.20 ± 2.50b	32.40 ± 2.08ab	32.08 ± 1.82ab	32.72 ± 1.56ab

Notes: Values are expressed as mean ± SEM for 24 eggs. Values with different lowercased and uppercased letters in the same row differ significantly ($P < .05$ and $P < .01$, respectively).

Table 4. Effects of supplementing the water offered Jinyun ducks with *B. subtilis* and PSB on the content of cholesterol and crude fat in eggs.

Items	Groups				
	C1	C2	T1	T2	T3
Cholesterol content (mg g ⁻¹)	14.44 ± 0.59	14.45 ± 0.21	14.66 ± 0.40	14.33 ± 0.68	14.83 ± 0.27
Crude fat content (%)	32.81 ± 0.34	32.47 ± 0.43	32.38 ± 0.21	32.04 ± 1.08	31.98 ± 0.57

Note: Values are expressed as mean ± SEM for nine eggs.

Table 5. Effects of supplementing the water offered Jinyun ducks with *B. subtilis* and PSB on activities of digestive enzymes in duodenum.

Items	Groups				
	C1	C2	T1	T2	T3
Trypsin (U mg protein ⁻¹)	414.74 ± 153.81	403.96 ± 47.35	510.02 ± 201.43	458.54 ± 54.07	370.37 ± 41.33
Lipase (U mg protein ⁻¹)	49.80 ± 25.23	52.65 ± 10.52	61.47 ± 5.90	60.04 ± 4.51	65.97 ± 7.11
Amylase (U mg protein ⁻¹)	2.36 ± 1.07	2.51 ± 1.07	2.22 ± 0.81	3.33 ± 0.56	2.90 ± 1.73

Note: Values are expressed as mean ± SEM for four ducks.

Egg quality was measured within 12 h of sampling. In this study, no significant differences were showed among the treatments in eggshell thickness, shape index, shell strength, yolk colour score and yolk weight. These results mean that the treatment of PSB alone or combined with *B. subtilis* has little effect on those indicators mentioned above. In a similar study, Ribeiro et al. (2014) investigated the effects of basal diet supplemented with *B. subtilis* on egg quality. Their findings suggested that *B. subtilis* had little effect on eggshell thickness, yolk or albumen weight. However, these findings were not consistent with Nahashon et al. (1994)'s, Mohan et al. (1995)'s and Xu et al. (2006)'s studies. Their studies suggested that *B. subtilis* can increase eggshell thickness.

Results showed that PSB alone treatment significantly improved the albumen height and Haugh unit, but high dose of *B. subtilis* has some bad effects on albumen height and Haugh unit. However, these findings were not consistent with Xu et al. (2006)'s studies, which suggested that *B. subtilis* can increase the Haugh unit.

4.3. Cholesterol and crude fat content in eggs

There was no significant difference ($P > .05$) in levels of cholesterol and crude fat content in eggs among all the treatments ($P > .05$) (Table 4).

There is a lot of research focusing on methods to reduce the levels of cholesterol in eggs and muscle because cholesterol is harmful for our health; this is especial true for old people. This study suggested that supplementation with PSB alone or with *B. subtilis* had little effect on the levels of cholesterol and

crude fat content in eggs. However, no other article has reported it before.

4.4. Digestive enzyme activity in duodenum

The concentrations of trypsin, lipase and amylase in a 10% homogenate of duodenum content samples in groups T1 and T2 were all greater than those of group C1, but showed no significant difference ($P > .05$) (Table 5).

This study suggested that the treatment of PSB alone or with *B. subtilis* has some beneficial effects on the trypsin, lipase and amylase activities, which means PSB alone or with *B. subtilis* can increase the body's metabolism. The increased egg production performance maybe related to the increased activity of trypsin, lipase and amylase. Lee et al. (2014) studied the effects of basal diet supplemented with 4.5 g kg⁻¹ *B. subtilis* on metabolic rate of pigs. This result was consistent with their study and their findings also suggested that *B. subtilis* can increase ($P < .05$) the coefficient of total tract apparent digestibility of dry matter, gross energy and crude protein.

4.5. Serum immunoglobulins

The concentrations of serum IgM showed no significant difference among the treatments ($P > .05$) (Table 6).

Compared to group C1, the concentrations of serum IgA enhanced by 33.40% ($P > .05$) in group T2, the concentrations of serum IgG enhanced by 20.22% ($P < .05$) in group C2 and by 22.47% ($P < .05$) in group T2 (Table 6).

Table 6. Effects of supplementing the water offered Jinyun ducks with *B. subtilis* and PSB on serum immunoglobulins.

Items	Groups				
	C1	C2	T1	T2	T3
IgA ($\mu\text{g mL}^{-1}$)	404.78 \pm 44.43	538.15 \pm 76.75	533.83 \pm 76.75	539.96 \pm 102.51	420.68 \pm 24.84
IgG (mg mL^{-1})	3.56 \pm 0.31a	4.28 \pm 0.30bc	3.79 \pm 0.33ab	4.36 \pm 0.47c	3.72 \pm 0.24ab
IgM ($\mu\text{g mL}^{-1}$)	291.82 \pm 19.19	339.48 \pm 34.62	321.99 \pm 19.61	343.36 \pm 61.41	304.22 \pm 6.05

Notes: Values are expressed as mean \pm SEM for 24 eggs for 4 ducks. Values with different lowercased letters in the same row differ significantly ($P < .05$).

Compared to group C1, the concentrations of serum IgA, IgG and IgM increased when the treatment of PSB alone or with *B. subtilis*, especially, the concentrations of serum IgG significantly increased ($P < .05$) in groups C2 and T2. Our results were consistent with Duc et al. (2004)'s and Salinas et al. (2005)'s studies. Their findings also suggested that *B. subtilis* is a non-pathogenic gram-positive bacterium and the probiotics can strength the body's humoral and cellular immune response. Some other articles also have reported that the use of *Lactobacillus spp.* alone or combined with *B. subtilis* can strength the cell or the humoral-mediated immune response in fish and piglets (Aly et al. 2008).

5. Conclusion

PSB combined with *B. subtilis* treatment significantly increased the laying rate, and decreased the feed-to-egg ratio in Jinyun ducks; further, PSB combined with *B. subtilis* treatment improved the albumen height, Haugh unit and the concentration of serum IgG; PSB alone treatment increased the yolk-to-egg ratio, albumen height and Haugh unit.

Disclosure statement

No potential conflict of interest was reported by the authors.

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