Effects of Bacillus coagulans on Production Performance, Egg Quality, and Plasma Biochemical Indices of Salmonella-Infected Laying Hens (Postprint)

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Abstract

This study aimed to investigate the effects of Bacillus coagulans on production performance and egg quality of laying hens before and after Salmonella infection, and to explore its influence on plasma biochemical indices in Salmonella-infected laying hens. A total of 400 Salmonella-negative, healthy Jinghong commercial laying hens were randomly allocated into 4 groups (Group A, B, C, and D) based on the principle of no significant difference in laying rate (P>0.05), with 5 replicates per group and 20 hens per replicate. The control groups (Group A and C) were fed a basal diet, while the Bacillus coagulans groups (Group B and D) received the basal diet supplemented with 2.5$×10^{10}$ CFU/kg Bacillus coagulans; after 1 week of feeding, Groups C and D were orally challenged with Salmonella enteritidis suspension (1$×10^{8}$ CFU/mL) quantitatively for 2 consecutive days, while Groups A and B received an equal volume of sterile phosphate-buffered saline (PBS), and the feeding trial continued for 3 weeks. The results showed that: 1) Before Salmonella infection, Bacillus coagulans had no significant effect on production performance of laying hens (P>0.05). After Salmonella infection, Salmonella significantly reduced the average daily feed intake of laying hens (P<0.05); Bacillus coagulans extremely significantly reduced the feed/egg ratio of laying hens (P<0.01), and significantly reduced average daily feed intake (P<0.05). 2) Before Salmonella infection, Bacillus coagulans extremely significantly increased the eggshell weight to egg weight ratio of laying hens (P<0.01). After Salmonella infection, neither Salmonella nor Bacillus coagulans had significant effects on egg quality parameters of laying hens (P>0.05). 3) After Salmonella infection, Bacillus coagulans significantly reduced plasma total cholesterol and triglyceride contents (P<0.05); Salmonella significantly reduced plasma total cholesterol, triglyceride, and calcium contents, significantly

increased plasma aspartate aminotransferase activity (P<0.05), and extremely significantly increased plasma alkaline phosphatase activity (P<0.01); Bacillus coagulans and Salmonella had significant interactive effects on plasma total protein content, calcium/phosphorus ratio, and aspartate aminotransferase activity (P<0.05). In conclusion, Bacillus coagulans can improve production performance and egg quality of laying hens, and exerts beneficial effects on protein metabolism, lipid metabolism, liver function, and calcium/phosphorus absorption to a certain extent.

Full Text

Effects of Bacillus coagulans on Performance, Egg Quality and Plasma Biochemical Parameters of Laying Hens Infected with Salmonella

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Abstract: This experiment was conducted to investigate the effects of *Bacillus coagulans* on the performance and egg quality of laying hens before and after *Salmonella* infection, and to explore its influence on plasma biochemical indices in *Salmonella*-infected laying hens. A total of 400 healthy, *Salmonella*-negative Jinghong commercial laying hens with similar laying rates (P>0.05) were randomly divided into four groups (A, B, C, and D) with five replicates per group and 20 hens per replicate. The control groups (A and C) were fed a basal diet, while the *Bacillus coagulans* groups (B and D) received the basal diet supplemented with 2.5$×10^{10}$ CFU/kg *Bacillus coagulans*. After one week of feeding, groups C and D were orally administered *Salmonella enterica* serovar Enteritidis suspension (1$×10^{8}$ CFU/mL) for two consecutive days, while groups A and B received an equivalent volume of sterile phosphate-buffered saline (PBS). The experiment continued for three additional weeks. The results showed: (1) Before *Salmonella* infection, *Bacillus coagulans* had no significant effect on hen performance (P>0.05). After infection, *Salmonella* significantly reduced average daily feed intake (P<0.05), while *Bacillus coagulans* significantly decreased feed-to-egg ratio (P<0.01) and average daily feed intake (P<0.05). (2) Before infection, *Bacillus coagulans* significantly increased eggshell weight to egg weight ratio (P<0.01). After infection, neither *Salmonella* nor *Bacillus coagulans* significantly affected egg quality parameters (P>0.05). (3) Post-infection, *Bacillus coagulans* significantly reduced plasma total cholesterol and triglyceride levels (P<0.05). *Salmonella* significantly decreased plasma total cholesterol, triglyceride, and calcium contents (P<0.05), significantly increased plasma aspartate aminotransferase activity (P<0.05), and markedly elevated plasma alkaline phosphatase activity (P<0.01). Significant interactions between *Bacillus coagulans* and *Salmonella* were observed for plasma total protein content, cal-

cium/phosphorus ratio, and aspartate aminotransferase activity (P<0.05). In conclusion, *Bacillus coagulans* can improve laying hen performance and egg quality, and demonstrates beneficial effects on protein metabolism, lipid metabolism, liver function, and calcium-phosphorus absorption.

Keywords: laying hens; *Bacillus coagulans*; *Salmonella*; performance

Salmonella enterica serovar Enteritidis (SE) is a major foodborne pathogen that causes disease in poultry and food poisoning in humans, potentially leading to death. Eggs represent a significant transmission vehicle for human *Salmonella* infection. In May 2010, a *Salmonella* outbreak in the United States linked to contaminated egg products resulted in the recall of 500 million eggs suspected of *Salmonella* contamination. Effective control of *Salmonella* in eggs is therefore critical for public health.

Recent studies have demonstrated that probiotics such as lactic acid bacteria, *Bifidobacterium*, and yeast can reduce *Salmonella* colonization in hosts. Mountzouris et al. [1] and Chen et al. [2] investigated the competitive exclusion capacity of probiotics against *Salmonella* and characterized their properties. Higgins et al. [3] reported that feeding *Lactobacillus* to *Salmonella*-challenged laying hens significantly reduced *Salmonella* positivity rates.

Bacillus coagulans is a Gram-positive bacterium and highly effective probiotic that enhances animal immunity, regulates intestinal microbial balance, and exhibits laxative, anti-diarrheal, and antioxidant effects. While numerous studies have investigated *B. coagulans* as a feed additive, research on its application in laying hens remains limited. This experiment was therefore designed to provide additional theoretical basis for the use of *B. coagulans* in laying hen production by examining its effects on performance, egg quality, and plasma biochemical indices in hens infected with *Salmonella enterica* serovar Enteritidis.

1.1 Experimental Materials

The *Salmonella enterica* serovar Enteritidis strain (CVCC3377) was purchased from the National Veterinary Microorganism Strain Collection Center. *Bacillus coagulans* ANCB161, isolated from chicken manure by our research group, contained 5.0$×10^{10}$ CFU/g viable bacteria.

1.2 Experimental Design and Management

Four hundred 45-week-old healthy Jinghong commercial laying hens were screened for *Salmonella* negativity using whole blood plate agglutination test and cloacal swab method. A 2$×$2 factorial design (with or without *B. coagulans* supplementation, with or without *Salmonella* challenge) was employed. Based on similar laying rates (P>0.05), hens were randomly allocated to four groups (A, B, C, D) with five replicates per group and 20 hens per replicate. Two groups were housed in each of two separate biosafety-level poultry houses with identical environmental conditions.

During the first experimental week, control groups (A and C) received the basal diet, while *B. coagulans* groups (B and D) received the basal diet supplemented with 2.5$×10^{10}$ CFU/kg *B. coagulans* to evaluate effects before *Salmonella* infection. After one week, hens in groups C and D were orally administered *S. enteritidis* suspension (1$×10^{8}$ CFU/mL) for two consecutive days, while groups A and B received equivalent sterile phosphate-buffered saline (PBS). The experiment continued for three additional weeks to assess the effects of *B. coagulans* on performance, egg quality, and serum biochemical indices post-infection.

The trial was conducted at the Zhuozhou Experimental Base of China Agricultural University. Hens were housed in individual cages in a closed poultry house with a 16-hour photoperiod at 16 lux intensity. Ambient temperature was maintained at 18–27°C with 50–70% relative humidity and longitudinal negative-pressure ventilation. Hens received mash feed ad libitum and water via nipple drinkers. The pre-trial period lasted two weeks, followed by a four-week formal experimental period. Dietary nutrient levels were formulated according to the Chinese "Feeding Standard of Chicken" (NY/T 33-2004) and Jinghong commercial layer management guidelines. The composition and nutrient levels of the basal diet are presented in Table 1 .

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

Notes: 1) The vitamin premix provided per kg of diet: VA 8,000 IU, VD_3 3,600 IU, VE 21 IU, VK_3 4.2 mg, VB_1 3 mg, VB_2 10.2 mg, folic acid 0.9 mg, calcium pantothenate 15 mg, nicotinic acid 45 mg, VB_6 5.4 mg, VB_{12} 0.024 mg, biotin 0.15 mg. 2) The trace elements premix provided per kg of diet: Cu (as copper sulfate) 8 mg, Fe (as ferrous sulfate) 60 mg, Zn 80 mg, Mn (as manganese sulfate) 80 mg, I (as potassium iodide) 0.6 mg, Se (as sodium selenite) 0.3 mg. 3) ME was a calculated value, while others were measured values.

1.4.1 Performance Parameters

Egg production and average egg weight were recorded per replicate to calculate laying rate and daily egg mass. Feed consumption and body weight were measured weekly per replicate to determine average daily feed intake and feed-to-egg ratio.

1.4.2 Egg Quality Assessment

Three eggs per replicate were collected weekly. Egg weight, albumen height, Haugh unit, and yolk color were measured using a SONOVA Egg Analyzer™ (Orka). Eggshell strength was determined using an Egg Force Reader (Orka), and eggshell thickness was measured with an Egg Shell Thickness Gauge (Orka). Yolk weight was recorded after separating from albumen, and eggshells were washed, dried, and weighed.

1.4.3 Plasma Biochemical Indices

At the end of the trial, one hen per replicate with body weight close to the replicate average was selected after 12 hours of feed withdrawal with water access. Blood samples were collected from wing veins into heparinized tubes, centrifuged at 3,500 rpm for 15 minutes to harvest plasma, which was stored at -80°C for analysis. Plasma total protein (TP), albumin (ALB), high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol (TC), triglycerides (TG), calcium (Ca), phosphorus (P), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (AKP) were measured using a Hitachi automatic biochemical analyzer with assay kits purchased from Sichuan Mike Biological Technology Co., Ltd.

1.5 Statistical Analysis

Data were processed using Excel 2007 and presented as means with pooled standard error of the mean (SEM). Performance and egg quality before *Salmonella* infection were analyzed by one-way ANOVA. Data after infection were analyzed using the general linear model (GLM) multivariate procedure in SPSS 18.0. Duncan' s multiple range test was used for pairwise comparisons. Significance was declared at $P<0.05$ and $P<0.01$ for significant and highly significant differences, respectively, with $0.05\leq P<0.10$ indicating a significant trend.

2.1 Effects of Bacillus coagulans on Performance of Laying Hens Before and After Salmonella Infection

As shown in Table 2 , before *Salmonella* infection, no significant differences were observed in performance between the control and *B. coagulans* groups ($P>0.05$), although *B. coagulans* tended to increase daily egg mass and decrease feed-to-egg ratio ($P<0.10$).

Table 2 Effects of *Bacillus coagulans* on performance of laying hens before *Salmonella* infection

Note: In the same row, values with different small letter superscripts differ significantly (P<0.05), while those with the same or no superscripts do not differ significantly (P>0.05). The same applies below.

Table 3 presents the performance data after *Salmonella* infection. No significant interaction was observed between *B. coagulans* and *Salmonella* on performance parameters ($P>0.05$). *Salmonella* significantly reduced average daily feed intake ($P<0.05$) but did not significantly affect other performance indices ($P>0.05$), although it tended to increase feed-to-egg ratio ($P<0.10$). *B. coagulans* significantly decreased average daily feed intake ($P<0.05$) and highly significantly reduced feed-to-egg ratio ($P<0.01$), without significantly affecting other performance parameters ($P>0.05$).

Table 3 Effects of *Bacillus coagulans* on performance of laying hens after *Salmonella* infection

2.2 Effects of Bacillus coagulans on Egg Quality of Laying Hens Infected with Salmonella

Table 4 shows that before *Salmonella* infection, *B. coagulans* significantly increased eggshell weight to egg weight ratio ($P<0.01$) compared with the control group, while other parameters showed no significant differences ($P>0.05$), although eggshell strength tended to increase ($P<0.10$).

Table 4 Effects of *Bacillus coagulans* on egg quality of laying hens before *Salmonella* infection

As shown in Table 5 , after *Salmonella* infection, no significant interaction existed between *B. coagulans* and *Salmonella* on egg quality parameters ($P>0.05$). *Salmonella* did not significantly affect egg quality ($P>0.05$) but tended to decrease yolk weight to egg weight ratio ($P<0.10$). *B. coagulans* also showed no significant effects on egg quality ($P>0.05$).

Table 5 Effects of *Bacillus coagulans* on egg quality of laying hens after *Salmonella* infection

2.3 Effects of Bacillus coagulans on Plasma Biochemical Indices of Laying Hens Infected with Salmonella

Table 6 reveals that after *Salmonella* infection, significant interactions between *B. coagulans* and *Salmonella* were detected for plasma total protein content, calcium/phosphorus ratio, and aspartate aminotransferase activity ($P<0.05$). *Salmonella* significantly reduced plasma total cholesterol, triglyceride, and calcium contents ($P<0.05$), significantly increased plasma aspartate aminotransferase activity ($P<0.05$), and highly significantly elevated plasma alkaline phosphatase activity ($P<0.01$). *Salmonella* did not significantly affect other plasma biochemical indices ($P>0.05$) but tended to increase total protein and globulin contents ($P<0.10$) and decrease LDL content and calcium/phosphorus ratio ($P<0.10$). *B. coagulans* significantly decreased plasma total cholesterol and triglyceride levels ($P<0.05$) without significantly affecting other indices ($P>0.05$), though it tended to increase plasma aspartate aminotransferase activity ($P<0.10$) and decrease plasma LDL content ($P<0.10$).

Table 6 Effects of *Bacillus coagulans* on plasma biochemical indices of laying hens after *Salmonella* infection

3.1 Effects of Bacillus coagulans on Performance of Laying Hens

Previous studies by Li Guojian [4] and Liu Lei et al. [5] demonstrated that appropriate probiotic supplementation could replace antibiotics and improve average daily gain and feed conversion efficiency in livestock and poultry. Hung et al. [6] reported that dietary *B. coagulans* supplementation improved feed conversion efficiency in broilers. Zhou et al. [7] found that dietary *B. coagulans* significantly enhanced the performance of Guangxi Yellow chickens. Consistent with these findings, our study showed that *B. coagulans* supplementation in

Salmonella-infected laying hens significantly increased feed intake and decreased feed-to-egg ratio, while also improving laying rate, average egg weight, and daily egg mass to some extent.

Our results also indicated that *Salmonella enteritidis* infection adversely affected growth performance, significantly reducing feed intake and increasing feed-to-egg ratio in laying hens. Dietary *B. coagulans* supplementation alleviated the performance decline caused by *Salmonella* infection. Wang Weimiao et al. [8] reported that *B. coagulans* exhibits antagonistic effects against intestinal pathogens such as *E. coli* and *Salmonella*. Collectively, these findings suggest that dietary *B. coagulans* supplementation may protect host health and mitigate pathogen stress during *Salmonella* infection.

3.2 Effects of Bacillus coagulans on Egg Quality of Laying Hens

Our study found that dietary *B. coagulans* supplementation did not significantly affect egg quality parameters before or after *Salmonella* infection, though it tended to improve eggshell thickness and strength. Xu et al. [9] reported that appropriate *Bacillus subtilis* supplementation increased eggshell thickness, yolk color, and Haugh unit. Li Junbo et al. [10] observed that *B. subtilis* improved egg weight, eggshell thickness, and Haugh unit, suggesting these improvements might be related to blood calcium levels and calcium utilization efficiency. Nahashon et al. [11] demonstrated that probiotics enhanced mineral absorption, particularly calcium and phosphorus, increasing serum levels and ensuring adequate calcium supply during egg formation.

3.3 Effects of Bacillus coagulans on Plasma Biochemical Indices of Laying Hens

Plasma biochemical indices are closely related to metabolism, nutritional status, and disease state in animals, and can reflect physiological and pathological changes. Probiotics such as *Bacillus licheniformis* and *B. subtilis* produce alkaline, neutral, or acidic proteases that promote dietary protein digestion and absorption [12]. Studies by Hua Jing et al. [13] and Chen Jiaxiang [14] showed that *B. subtilis* or *B. licheniformis* supplementation in broilers increased plasma total protein and albumin contents. Our findings are consistent with these reports, showing a significant interaction between *B. coagulans* and *Salmonella* on plasma total protein, with *B. coagulans* significantly increasing plasma total protein post-infection, reflecting host stress responses to the interaction between *B. coagulans* and *Salmonella*.

Plasma cholesterol and triglycerides are primary indicators of lipid metabolism. Lü Zunzhou et al. [15] found that *Bacillus* supplementation significantly reduced plasma triglyceride content. Intestinal microorganisms play important roles in lipid circulation by converting free fatty acids into various triglyceride forms, thereby preventing triglyceride entry into blood. Plasma cholesterol content is influenced by HMG-CoA reductase, a key enzyme in cholesterol synthesis.

Probiotics can inhibit this key enzyme, suggesting *Bacillus* may reduce serum cholesterol by suppressing HMG-CoA reductase activity [16]. Our study showed that *B. coagulans* supplementation significantly reduced plasma total cholesterol and triglyceride levels, consistent with Li Fubin et al. [17], who reported that *B. licheniformis* decreased serum cholesterol.

Plasma alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase are important clinical indicators for evaluating normal liver, pancreatic, and kidney function. Our results showed that *Salmonella* infection significantly increased plasma aspartate aminotransferase and alkaline phosphatase activities, though the underlying mechanisms require further investigation.

Additionally, *B. coagulans* supplementation increased plasma calcium and phosphorus contents, indicating enhanced mineral absorption, possibly through organic acids produced by *B. coagulans* metabolism. Xu Haiyan et al. [18] found that *Bacillus* fermentation broth contains acetic, propionic, and butyric acids that lower intestinal pH and promote mineral absorption.

Conclusions:

1. *Salmonella enteritidis* infection significantly reduced average daily feed intake and increased feed-to-egg ratio in laying hens, but did not significantly affect other performance parameters or egg quality. In plasma, *Salmonella* significantly decreased total cholesterol, triglyceride, and calcium contents, significantly increased aspartate aminotransferase and alkaline phosphatase activities, and tended to reduce calcium and phosphorus levels.
2. Dietary *Bacillus coagulans* supplementation significantly decreased feed-to-egg ratio and average daily feed intake in *Salmonella*-infected laying hens, improved other performance parameters, but did not significantly affect egg quality. *B. coagulans* significantly reduced plasma total cholesterol and triglyceride contents while improving plasma protein and lipid metabolism.

References:

[1] MOUNTZOURIS K C, BALASKAS C, XANTHAKOS I, et al. Effects of a multi-species probiotic on biomarkers of competitive exclusion efficacy in broilers challenged with *Salmonella enteritidis* [J]. *British Poultry Science*, 2009, 50(4): 467–478.

[2] CHEN C Y, TSEN H Y, LIN C L, et al. Oral administration of a combination of select lactic acid bacteria strains to reduce the *Salmonella* invasion and inflammation of broiler chicks [J]. *Poultry Science*, 2012, 91(9): 2139–2147.

[3] HIGGINS S E, HIGGINS J P, WOLFENDEN A D, et al. Evaluation of a *Lactobacillus*-based probiotic culture for the reduction of *Salmonella enteritidis* in neonatal broiler chicks [J]. *Poultry Science*, 2008, 87(1): 27–31.

[4] LI Guojian. Effects of *Bacillus coagulans* as antibiotic substitute on pig performance [J]. *Henan Agricultural Sciences*, 2004, 33(10): 72-74.

[5] LIU Lei, ZHU Lixian. Effects of *Sporolactobacillus* on growth performance, intestinal development and microbial flora of broilers [J]. *Chinese Journal of Animal Nutrition*, 2011, 23(12): 2136-2142.

[6] HUNG A T, LIN S Y, YANG T Y, et al. Effects of *Bacillus coagulans* ATCC 7050 on growth performance, intestinal morphology, and microflora composition in broiler chickens [J]. *Animal Production Science*, 2012, 52(9): 874-879.

[7] ZHOU X, WANG Y, GU Q, et al. Effect of dietary probiotic, *Bacillus coagulans*, on growth performance, chemical composition, and meat quality of Guangxi Yellow chicken [J]. *Poultry Science*, 2010, 89(3): 588-593.

[8] WANG Weimiao, PAN Ling, ZHOU Jie, et al. *In vitro* antibacterial test of lactic acid bacteria from silage and bacteriocin extraction [J]. *Feed Review*, 2006(4): 1-3.

[9] XU C L, JI C, MA Q, et al. Effects of a dried *Bacillus subtilis* culture on egg quality [J]. *Poultry Science*, 2006, 85(2): 364-368.

[10] LI Junbo, CHENG Tingshui, LÜ Wuxing, et al. Effects of *Bacillus subtilis* preparation on performance, egg quality and nutrient digestibility in laying hens [J]. *China Poultry*, 2009, 31(4): 15-17.

[11] NAHASHON S N, NAKAUE H S, MIROSH L W. Production variables and nutrient retention in Single Comb White Leghorn laying pullets fed diets supplemented with direct-fed microbials [J]. *Poultry Science*, 1994, 73(11): 1699-1711.

[12] ZHANG Z F, KIM I H. Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers [J]. *Poultry Science*, 2014, 93(2): 364-370.

[13] HUA Jing, GUO Yuqin, ZHANG Shuping, et al. Effects of dietary *Bacillus subtilis* on average daily gain and blood biochemical indices in broilers [J]. *Heilongjiang Animal Science and Veterinary Medicine*, 2003(2): 14-15.

[14] CHEN Jiaxiang, ZHANG Renyi, WANG Quanxi, et al. Effects of *Bacillus licheniformis* on growth performance, antioxidant indices and blood biochemical parameters in broilers [J]. *Chinese Journal of Animal Nutrition*, 2010, 22(4): 1019-1023.

[15] LÜ Zunzhou, YUAN Xiaoxiao, HAO Zhu, et al. Effects of *Bacillus* preparation on laying performance, serum indices and intestinal flora of laying hens [J]. *Chinese Journal of Veterinary Science*, 2012, 32(7): 1068-1072.

[16] KNAP I, KEHLET A B, BENNEDSEN M, et al. *Bacillus subtilis* (DSM17299) significantly reduces *Salmonella* in broilers [J]. *Poultry Science*, 2011, 90(8): 1690-1694.

[17] LI Fubin, CHEN Baojiang, LIANG Chenchong, et al. Effects of *Bacillus licheniformis* on performance, egg quality and serum indices in laying hens [J]. *China Feed*, 2010, 33(13): 5-8.

[18] XU Haiyan, CAO Bin, ZHANG Zhiyan, et al. Study on fermentation metabolites of *Bacillus* [J]. *Feed China*, 2006(9): 22-23.

Note: Figure translations are in progress. See original paper for figures.