Effects of Bacillus subtilis on Production Performance and Eggshell Quality of Laying Hens in the Late Laying Period (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary Bacillus subtilis supplementation on production performance and eggshell quality of laying hens during the late laying period. A total of 420 healthy 52-week-old Hy-Line Brown laying hens with similar laying rates and body weights were selected and randomly divided into 4 groups with 7 replicates per group and 15 hens per replicate. The control group was fed a corn-soybean meal basal diet, and the experimental groups were supplemented with 200, 400, and 800 mg/kg Bacillus subtilis in the basal diet, respectively. The pre-trial period was 1 week, and the formal trial period was 12 weeks. The results showed that, compared with the control group: 1) Dietary supplementation with 200, 400, and 800 mg/kg Bacillus subtilis had no significant effect on laying rate and average egg weight ($P>0.05$), but significantly reduced average daily feed intake ($P<0.05$); supplementation with 400 and 800 mg/kg Bacillus subtilis significantly reduced feed-to-egg ratio ($P<0.05$). 2) Dietary supplementation with 200, 400, and 800 mg/kg Bacillus subtilis significantly increased eggshell thickness ($P<0.05$) and significantly reduced broken and soft egg rate ($P<0.05$); supplementation with 800 mg/kg Bacillus subtilis significantly improved eggshell strength and shell weight ratio ($P<0.05$). 3) Dietary supplementation with 800 mg/kg Bacillus subtilis improved the ultrastructure of eggshell and significantly increased calcium content in eggshell ($P<0.05$). It can be concluded that dietary Bacillus subtilis supplementation can improve production performance, enhance eggshell quality, and reduce broken and soft egg rate in laying hens during the late laying period. Under the conditions of this experiment, dietary supplementation with 800 mg/kg Bacillus subtilis is recommended.

Full Text

Effects of Bacillus subtilis on Performance and Eggshell Quality of Laying Hens during Later Period of Laying

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Abstract: This experiment was conducted to investigate the effects of dietary Bacillus subtilis supplementation on performance and eggshell quality of laying hens during the later production period. Four hundred and twenty healthy 52-week-old Hy-Line brown laying hens with similar egg production rates and body weights were randomly allocated into 4 groups with 7 replicates per group and 15 hens per replicate. The control group received a corn-soybean meal basal diet, while the experimental groups received the basal diet supplemented with 200, 400, or 800 mg/kg Bacillus subtilis. A 1-week pre-trial period was followed by a 12-week experimental period. The results showed that, compared with the control group: (1) supplementation with 200, 400, and 800 mg/kg Bacillus subtilis had no significant effects on egg production rate or average egg weight ($P>0.05$), but significantly reduced average daily feed intake ($P<0.05$); supplementation with 400 and 800 mg/kg significantly decreased the feed-to-egg ratio ($P<0.05$). (2) Supplementation with 200, 400, and 800 mg/kg significantly increased eggshell thickness ($P<0.05$) and significantly reduced broken/soft egg rate ($P<0.05$); the 800 mg/kg group showed significantly higher eggshell strength and eggshell percentage ($P<0.05$). (3) The 800 mg/kg supplementation improved eggshell ultrastructure and significantly increased eggshell calcium content ($P<0.05$). In conclusion, dietary Bacillus subtilis supplementation can improve performance, enhance eggshell quality, and reduce broken/soft egg rates in laying hens during the later production period. Under the conditions of this experiment, 800 mg/kg Bacillus subtilis is recommended as the optimal supplementation level.

Keywords: Bacillus subtilis; laying hens; performance; eggshell quality

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China is currently the world's largest egg producer and consumer, accounting for approximately 38% of global production and consumption. With the development of large-scale, intensive, and mechanized egg production, eggshell quality issues have become increasingly prominent. Statistics indicate that broken and soft-shelled eggs account for 6%–13% of total egg production in commercial layers, causing annual economic losses of 500 million RMB in China. As hens age, their ability to digest and absorb nutrients declines, leading to reduced perfor-

mance. Additionally, egg weight increases while shell thickness decreases in later production stages, with broken and soft egg rates exceeding 20%, representing a serious quality challenge. These issues not only raise consumer concerns but also cause substantial losses to the egg industry and processing sector, affecting the healthy development of the poultry industry. Therefore, identifying effective measures to improve eggshell quality, particularly for late-stage laying hens, holds significant practical importance.

Bacillus subtilis is a probiotic additive—an endospore-forming Gram-positive aerobic bacterium with excellent heat and acid-alkali resistance, widely used in animal production. Its spore form can withstand temperature and pressure changes during feed processing and storage without consuming feed nutrients, while also tolerating gastric acid and bile salts to maintain high viability in the digestive tract, where it germinates into active cells under suitable conditions. Bacillus subtilis promotes proliferation of beneficial bacteria and inhibits pathogen growth in the intestinal tract, improving microbial balance. It increases small intestinal villus height, enhances gut integrity, and improves nutrient absorption and utilization, while reducing ammonia and nitrogen excretion and decreasing harmful gas emissions in poultry houses. Previous studies have reported that dietary Bacillus subtilis supplementation increased egg production rate by 4.4% and reduced feed-to-egg ratio by 0.5% in laying hens, thereby improving performance. Other research demonstrated that Bacillus subtilis can increase eggshell thickness and strength while reducing broken/soft egg rates. Although numerous studies have investigated Bacillus subtilis in laying hen production, its effects and mechanisms on performance and eggshell quality during the later production period remain poorly documented. Therefore, this study used late-stage Hy-Line brown laying hens to investigate the effects and mechanisms of dietary Bacillus subtilis supplementation on performance and eggshell quality, providing theoretical support for practical application.

1.1 Experimental Material

Bacillus subtilis powder was provided by Shanghai Muguang Enterprise Development Co., Ltd., containing 1.0×10^{1} CFU/g viable bacteria.

1.2 Experimental Design and Diets

A single-factor experimental design was employed. Four hundred and twenty healthy 52-week-old Hy-Line brown laying hens with similar egg production rates and body weights were randomly divided into 4 groups with 7 replicates per group and 15 hens per replicate. Hens were housed in tiered cages (47 cm × 37 cm × 47 cm) with 3 hens per cage. The control group received a corn-soybean meal basal diet, while the experimental groups received the basal diet supplemented with 200, 400, or 800 mg/kg Bacillus subtilis (providing 2×10 , 4×10 , and 8×10 CFU/g, respectively). The basal diet was formulated according to NRC (1994), Chinese Feeding Standard of Chickens (NY/T 33–

2004), and the Hy-Line brown laying hen management manual. The composition and nutrient levels of the basal diet are shown in Table 1 .

1.3 Management

Hens were raised in a semi-open house with three-tier cage systems. Lighting consisted of natural light plus artificial supplementation to provide 16 h/d at 20 lx intensity, with longitudinal negative-pressure ventilation. Feed was provided manually three times daily (06:30, 13:00, and 17:30). Eggs were collected at 15:00 daily. Hens had free access to feed and water. The house was disinfected weekly, and conventional immunization protocols were followed.

1.4 Measurements

1.4.1 Performance During the experimental period, daily egg number and weight were recorded per replicate to calculate average egg weight and egg production rate. Average daily feed intake and feed-to-egg ratio were calculated every two weeks per replicate.

1.4.2 Eggshell Quality At the end of week 12, for three consecutive days, six eggs per replicate with weights close to the average (excluding broken and soft-shelled eggs) were selected daily to measure egg quality and eggshell apparent quality. Intact eggshells were retained for ultrastructure observation and calcium/phosphorus content determination.

Eggshell apparent quality measurement: Eggshell weight was measured using an analytical balance. Eggshell thickness was measured using an Eggshell Thickness Gauge (Orka Technology Ltd.), and eggshell strength was measured using an Egg Force Reader (Orka Technology Ltd.). Eggshell percentage was calculated as: Eggshell percentage (%) = 100 × eggshell weight / egg weight.

Eggshell ultrastructure observation: A JEOL 6301F field emission scanning electron microscope was used to observe eggshell ultrastructure. Eggshell surface grease was removed with alcohol, and small samples were prepared and mounted on specimen stages with conductive tape. After gold sputtering, samples were placed in the microscope chamber and observed/photographed at various magnifications under high vacuum.

Eggshell calcium and phosphorus content determination: Eggshells were cleaned with warm distilled water, dried, and ground. Calcium content was determined by potassium permanganate method and phosphorus content by colorimetric method.

1.5 Statistical Analysis

Data were processed using Excel 2010 and analyzed by one-way ANOVA using SPSS 19.0 software. Duncan' s multiple range test was used for intergroup

comparisons. Significance was declared at $P<0.05$. Results are expressed as "mean ± standard deviation."

2.1 Effects of Bacillus subtilis on Performance of Laying Hens during Later Period of Laying

As shown in Table 2 , dietary supplementation with different levels of Bacillus subtilis had no significant effects on egg production rate or average egg weight ($P>0.05$). However, all three experimental groups showed significantly lower average daily feed intake compared with the control group ($P<0.05$). The feed-to-egg ratio was also lower in all experimental groups, with the 400 and 800 mg/kg groups being significantly lower than the control ($P<0.05$). These results suggest that dietary supplementation with 400 and 800 mg/kg Bacillus subtilis improved laying hen performance.

2.2 Effects of Bacillus subtilis on Eggshell Quality of Laying Hens during Later Period of Laying

Table 3 shows that, compared with the control group, dietary supplementation with different levels of Bacillus subtilis significantly increased eggshell thickness ($P<0.05$) and significantly reduced broken/soft egg rate ($P<0.05$), though no significant differences were observed among the three experimental groups ($P>0.05$). Eggshell strength in all experimental groups was higher than the control, with the 800 mg/kg group being significantly higher ($P<0.05$). Eggshell strength increased linearly with supplementation level ($P<0.05$). While eggshell weight tended to increase with Bacillus subtilis supplementation, no significant differences were detected among groups ($P>0.05$). The eggshell percentage was higher in all experimental groups than the control, with the 400 and 800 mg/kg groups showing significant increases ($P<0.05$). These results indicate that dietary supplementation with 400 and 800 mg/kg Bacillus subtilis improved eggshell apparent quality.

Since the 800 mg/kg group showed significantly higher eggshell thickness and strength than the control ($P<0.05$), ultrastructure observations were conducted for these two groups. Figure 1 [Figure 1: see original paper] reveals that the 800 mg/kg group exhibited more numerous and densely arranged mammillary layer cones with smaller cone heights and gaps, whereas the control group showed fewer cones with irregular shapes and arrangement. Additionally, at the same magnification, the 800 mg/kg group demonstrated greater effective thickness and total thickness, indicating that Bacillus subtilis can improve eggshell ultrastructure.

Table 4 shows that eggshell phosphorus content did not differ significantly among groups ($P>0.05$), but calcium content in the 800 mg/kg group was significantly higher than the control ($P<0.05$), suggesting enhanced calcium deposition in eggshells.

3.1 Effects of Dietary Bacillus subtilis Supplementation on Performance of Late-Stage Laying Hens

Previous studies have reported that dietary Bacillus subtilis supplementation can improve animal performance, though findings have been inconsistent. Wang et al. found that adding 1×10^{1} CFU/g Bacillus subtilis to diets of 54- and 60-week-old Hy-Line brown hens reduced average daily feed intake and feed-to-egg ratio, but differences were not significant. Other studies reported that supplementation with 1×10 and 8×10 CFU/g had no significant effects on these parameters. However, research by Ding and Xu et al. demonstrated that 3×10 , 6×10 , and 9×10 CFU/g significantly reduced average daily feed intake and feed-to-egg ratio without affecting egg production rate, thereby improving performance. These discrepancies may be related to supplementation levels, breed differences, and age variations.

In this study, dietary supplementation with 200–800 mg/kg (2×10 -8×10 CFU/g) Bacillus subtilis reduced average daily feed intake, with the 400 and 800 mg/kg (4×10 and 8×10 CFU/g) levels significantly decreasing the feed-to-egg ratio. This may be attributed to Bacillus subtilis consuming excess oxygen in the gut, producing bacteriocins and volatile antimicrobial substances that create a favorable environment for beneficial bacteria like Lactobacillus and Bifidobacterium, thereby lowering intestinal pH and inhibiting harmful bacteria to regulate microecological balance. Bacillus subtilis also improves gut integrity and enhances small intestinal digestive and absorptive functions. It produces various digestive enzymes and beneficial metabolites such as lactic acid, acetic acid, and propionic acid, which synergistically promote endogenous enzyme activity, facilitating nutrient degradation and improving feed conversion efficiency, thus reducing feed intake and feed-to-egg ratio.

3.2 Effects of Dietary Bacillus subtilis Supplementation on Eggshell Quality of Late-Stage Laying Hens

Eggshell quality comprises structural and material properties, both influencing overall quality. Structural properties include thickness, strength, percentage, and ultrastructure, while material properties mainly involve organic and inorganic components. Eggshell contains approximately 1.6% water, 3.3%–3.5% organic matter, and about 95% inorganic minerals—primarily calcium carbonate (94%), with the remainder being magnesium carbonate and calcium phosphate. Eggshell calcium content is approximately 37.5%, with 60%–75% derived from the diet and the remainder from medullary bone mobilization.

Improving eggshell quality is a critical issue in layer production and a major research focus worldwide. Dietary Bacillus subtilis supplementation has been shown to increase eggshell thickness and strength, improve quality, reduce broken/soft egg rates, and enhance economic benefits. Wang reported that Bacillus subtilis improved eggshell apparent quality and ultrastructure in late-stage laying hens (post-54 weeks) without significantly affecting albumen height or

Haugh unit. The current results demonstrate that all supplementation levels significantly increased eggshell thickness and reduced broken/soft egg rates, with the 800 mg/kg group showing significantly higher eggshell strength and percentage. Some studies have found that Bacillus subtilis improves performance but not eggshell quality, possibly due to differences in breed, age, and experimental duration. In this study, eggshell phosphorus content did not differ among groups, but calcium content was significantly higher in the 800 mg/kg group, indicating increased calcium deposition.

The mechanism by which Bacillus subtilis improves eggshell quality remains unclear, but reduced eggshell quality in aging hens is associated with decreased intestinal calcium absorption efficiency. Bacillus subtilis may enhance nutrient absorption, particularly calcium and phosphorus, thereby improving eggshell quality. Calcium and phosphorus absorption efficiency is pH-dependent, as increased acidity promotes calcium salt dissolution and absorption. Bacillus subtilis may lower intestinal pH by promoting Lactobacillus growth and producing lactic acid metabolites, thus enhancing calcium absorption. Research has shown that probiotics promote dietary calcium absorption in laying hens, and improved calcium utilization is a primary mechanism for enhanced eggshell quality.

The ultrastructure observations revealed that, compared with the control, the 800 mg/kg group showed more numerous mammillary cones with smaller heights, widths, and gaps, and more uniform, densely packed crystals within the eggshell, while the control group exhibited fewer irregularly arranged cones. This aligns with previous findings that dense mammillary layer arrangement effectively prevents crack initiation and propagation, demonstrating that Bacillus subtilis improves eggshell quality by enhancing microstructure.

4 Conclusion

1. Dietary supplementation with 400 and 800 mg/kg Bacillus subtilis had no significant effects on egg production rate or average egg weight but significantly reduced average daily feed intake, feed-to-egg ratio, and broken/soft egg rate in late-stage laying hens.
2. Dietary supplementation with 800 mg/kg Bacillus subtilis significantly increased eggshell thickness, eggshell strength, and eggshell calcium content while improving eggshell ultrastructure.

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