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

Authors: Cui Chuangfei, Wang Jing, Guanghai Qi, Zhang Haijun, Wu Shugeng, Shi Zhaoguo

Date: 2018-12-24T00:00:00+00:00

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 allocated into 4 groups with 7 replicates per group and 15 hens per replicate. The control group was fed a corn-soybean meal basal diet, while the experimental groups were supplemented with 200, 400, and 800 mg/kg Bacillus subtilis to the basal diet, respectively. The preliminary period lasted for 1 week, and the experimental period lasted for 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 ($P<0.05$); dietary supplementation with 400 and 800 mg/kg Bacillus subtilis significantly reduced the 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 the broken and soft egg rate ($P<0.05$); dietary 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 ($P<0.05$). These results indicate 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

CUI Chuangfei[1],[2], WANG Jing[2], QI Guanghai[2], ZHANG Haijun[2], WU Shugeng[2]*, SHI Zhaoguo[1]*

[1]College of Animal Science and Technology, Gansu Agricultural University, Lanzhou 730070, China
[2]Key Laboratory of Feed Biotechnology of Ministry of Agriculture, National Engineering Research Center of Biological Feed, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China

Abstract: This experiment investigated the effects of dietary Bacillus subtilis supplementation on performance and eggshell quality of laying hens during the later laying 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 to 4 groups with 7 replicates per group and 15 hens per replicate. The control group received a corn-soybean meal basal diet, while 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. Compared with the control group: (1) supplementation with 200, 400, and 800 mg/kg Bacillus subtilis had no significant effect 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 reduced feed-to-egg ratio ($P<0.05$). (2) All supplementation levels significantly increased eggshell thickness ($P<0.05$) and significantly decreased broken/soft egg rate ($P<0.05$); the 800 mg/kg group significantly increased eggshell strength and eggshell percentage ($P<0.05$). (3) The 800 mg/kg supplementation improved eggshell ultrastructure and significantly increased eggshell calcium content ($P<0.05$). These results indicate that dietary Bacillus subtilis can improve performance, enhance eggshell quality, and reduce broken/soft egg rates in late-phase laying hens. Under the conditions of this study, 800 mg/kg Bacillus subtilis is recommended as the optimal supplementation level.

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

---

Introduction

China is currently the world' s largest egg producer and consumer, accounting for approximately 38% of global production and consumption. However, with the intensification, scaling, and mechanization of egg production, eggshell quality issues have become increasingly prominent. Statistics indicate that broken and soft-shelled eggs comprise 6%–13% of total egg production, causing annual economic losses of 500 million RMB in China. As hens age, their ability to

digest and absorb nutrients declines, leading to reduced performance. Additionally, increased egg weight and thinner shells in older hens result in broken/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-phase 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 tolerates temperature and pressure variations during feed processing and storage without consuming feed nutrients, while surviving gastric acid and bile salts to maintain high viability in the digestive tract, germinating into active cells under suitable conditions. Bacillus subtilis promotes proliferation of beneficial bacteria in the hen intestine, inhibits pathogen growth, and improves microbial flora. It increases small intestinal villus height, enhances gut integrity, and improves nutrient absorption and utilization while reducing ammonia and nitrogen excretion and harmful gas emissions in poultry houses. Previous studies reported that dietary Bacillus subtilis increased egg production rate by 4.4% and reduced feed-to-egg ratio by 0.5%, thereby improving performance. Other research demonstrated that Bacillus subtilis increased eggshell thickness and strength while reducing broken/soft egg rates. Although numerous studies have examined Bacillus subtilis in laying hens, its effects and mechanisms on performance and eggshell quality during the late laying period remain poorly documented. This study therefore investigated the effects and underlying mechanisms of dietary Bacillus subtilis on performance and eggshell quality in late-phase Hy-Line Brown laying hens to provide theoretical support for practical application.

Materials and Methods

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 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

management manual. Basal diet composition and nutrient levels are presented in Table 1 .

1.3 Management

Hens were raised in a semi-open poultry house with three-tier cage systems. Lighting consisted of natural light plus artificial supplementation to achieve 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 ad libitum access to feed and water. Disinfection was performed weekly, and conventional immunization protocols were followed.

1.4 Sample Collection and Measurements

1.4.1 Performance Metrics 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 biweekly 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 or soft-shelled eggs) were selected daily to determine egg quality and eggshell characteristics. Intact eggshells were retained for ultrastructure observation and calcium/phosphorus content analysis.

Eggshell quality measurements: Eggshell weight was determined using an analytical balance. Eggshell thickness was measured with an Egg Shell Thickness Gauge (Orka Technology Ltd.), and eggshell strength was measured with an Egg Force Reader (Orka Technology Ltd.). Eggshell percentage was calculated as: (eggshell weight/egg weight) $\times$ 100.

Eggshell ultrastructure observation: A JEOL 6301F field emission scanning electron microscope was used to examine eggshell ultrastructure. Eggshell surface grease was removed with alcohol before preparing small specimens mounted on sample 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: Eggshells were cleaned with warm distilled water, dried, and pulverized. 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. Duncan' s multiple range test was used for intergroup comparisons. Significance was set at $P<0.05$. Results are expressed as means $\pm$ standard deviation.

Results

2.1 Effects of Bacillus subtilis on Performance of Late-Phase Laying Hens

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

2.2 Effects of Bacillus subtilis on Eggshell Quality of Late-Phase Laying Hens

Table 3 shows that compared with the control group, all Bacillus subtilis supplementation levels 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 exceeded that of the control group, with the 800 mg/kg group showing a significant increase ($P<0.05$). Eggshell strength increased linearly with supplementation level ($P<0.05$). While eggshell weight tended to increase with supplementation, no significant intergroup differences were observed ($P>0.05$). Eggshell percentage in all experimental groups exceeded that of the control group, with significant increases in the 400 and 800 mg/kg groups ($P<0.05$). These results indicate that dietary supplementation with 400 and 800 mg/kg Bacillus subtilis improved eggshell quality.

Since the 800 mg/kg group showed significantly greater eggshell thickness and strength than the control group ($P<0.05$), ultrastructure was compared between these 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 interspaces, whereas the control group showed fewer, irregularly shaped and arranged cones. Furthermore, at the same magnification, the 800 mg/kg group demonstrated greater effective thickness and total thickness, indicating that Bacillus subtilis improved 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 in the control group ($P<0.05$), suggesting enhanced calcium deposition in eggshells.

Discussion

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

Previous studies have reported that dietary Bacillus subtilis can improve animal performance, though findings vary. Wang (2015) supplemented 54- and 60-week-old Hy-Line Brown hens with 1×10^{1} CFU/g Bacillus subtilis and observed reduced average daily feed intake and feed-to-egg ratio compared with controls, though differences were not significant. Other studies found that 1×10 and 8×10 CFU/g Bacillus subtilis had no significant effect on these parameters. In contrast, Ding (2013) and Xu et al. (2006) reported 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 relate to differences in supplementation levels, hen breeds, and ages.

In this study, dietary Bacillus subtilis at 200–800 mg/kg (2×10 -8×10 CFU/g) reduced average daily feed intake, with 400 and 800 mg/kg (4×10 and 8×10 CFU/g) significantly reducing feed-to-egg ratio. This may occur because Bacillus subtilis consumes excess oxygen in the intestine, produces bacteriocins and volatile antimicrobial substances, creating an environment favorable for beneficial bacteria such as Lactobacillus and Bifidobacterium, thereby lowering intestinal pH and inhibiting harmful bacteria to modulate microecological balance. Bacillus subtilis also improves gut integrity and enhances small intestinal digestive-absorptive function, producing various digestive enzymes and beneficial metabolites including lactic, acetic, and propionic acids that synergistically promote endogenous enzyme activity. This facilitates degradation of feed nutrients, improves feed conversion efficiency, and consequently reduces feed intake and feed-to-egg ratio.

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

Eggshell properties comprise structural and material attributes, both influencing quality. Structural attributes include thickness, strength, percentage, and ultrastructure, while material attributes primarily comprise organic and inorganic components. Eggshell contains approximately 1.6% water, 3.3–3.5% organic matter, and about 95% inorganic minerals, mainly calcium carbonate (~94%) with smaller amounts of 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 resorption.

Improving eggshell quality represents a critical issue in egg production and an active 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 (2015) found that Bacillus subtilis improved eggshell quality and ultrastructure in late-

phase hens (post-54 weeks) without significantly affecting albumen height or Haugh unit. Our results demonstrate that all Bacillus subtilis levels significantly increased eggshell thickness and reduced broken/soft egg rates, with the 800 mg/kg group significantly improving eggshell strength and percentage. Some studies reported that Bacillus subtilis improved performance without significantly affecting eggshell quality, possibly due to differences in hen breed, age, and experimental duration. In this study, eggshell phosphorus content did not differ among groups, but the 800 mg/kg group showed significantly higher calcium content, indicating increased calcium deposition.

The mechanism by which Bacillus subtilis improves eggshell quality remains unclear, but reduced eggshell quality in aging hens correlates with decreased intestinal calcium absorption efficiency. Bacillus subtilis may enhance intestinal absorption of nutrients, particularly calcium and phosphorus, thereby improving eggshell quality. Calcium and phosphorus absorption efficiency relates to intestinal pH, with increased acidity promoting calcium salt dissolution and absorption. Bacillus subtilis may reduce intestinal pH by promoting Lactobacillus growth and producing lactic acid metabolites, thereby enhancing calcium absorption efficiency. Research indicates that probiotics promote dietary calcium absorption in laying hens, and improved calcium utilization represents the primary mechanism for eggshell quality improvement.

Our ultrastructure analysis revealed that compared with controls, the 800 mg/kg group showed more numerous mammillary cones with smaller heights, widths, and interspaces, and more uniform, densely packed crystals within the eggshell, whereas control shells exhibited fewer, irregularly arranged cones. This aligns with Wang (2015), as dense mammillary layer arrangement effectively prevents crack initiation and propagation, demonstrating that Bacillus subtilis improves eggshell quality through ultrastructural enhancement.

Conclusion

Dietary supplementation with 400 and 800 mg/kg Bacillus subtilis had no significant effect on egg production rate or average egg weight but significantly reduced average daily feed intake, feed-to-egg ratio, and broken/soft egg rate. Supplementation with 800 mg/kg Bacillus subtilis significantly increased eggshell thickness and strength, enhanced eggshell calcium content, and improved eggshell ultrastructure.

References

[1] ROLAND D A. Research note: egg shell problems: estimates of incidence and economic impact[J]. Poultry Science, 1988, 67(12): 1801-1803.

[2] 宋慧芝, 王俊, 叶均安. 鸡蛋蛋壳受载特性的有限元研究 [J]. 浙江大学学报（农业与生命科学版）, 2006, 32(3): 350-354.

[3] SCHEIDELER S E, AL-BATSHAN H. Basics of calcium, phosphorus nutrition in layers studied[J]. Feedstuffs (USA), 1994, 66(14): 15-16.

[4] ROLAND D A. Further studies of effects of sodium aluminosilicate on egg shell quality[J]. Poultry Science, 1988, 67(4): 577-584.

[5] Nys Y. Recent developments in layer nutrition for optimising shell quality[C]//Proceedings of 13th European Symposium on Poultry Nutrition. Blankenberge, Belgium: [s.n.], 2001: 42-52.

[6] 王晓阁. 枯草芽孢杆菌研究进展与展望 [J]. 中山大学研究生学刊（自然科学与医学版）, 2012, 33(3): 14-23.

[7] CENCI G, TROTTA F, CALDINI G. Tolerance to challenges mimicking gastrointestinal transit spores vegetative cells Bacillus clausii[J]. Journal of Applied Microbiology, 2006, 101(6): 1208-1215.

[8] OH J K, PAJARILLO E A B, CHAE J P, et al. Protective effects of Bacillus subtilis against Salmonella infection in the microbiome of Hy-Line brown layers[J]. Asian-Australasian Journal of Animal Sciences, 2017, 30(9): 1332-1339.

[9] 张爱武, 薛军. 枯草芽孢杆菌在动物生产中的应用效果 [J]. 中国畜牧兽医, 2011, 38(4): 234-238.

[10] 马春阳, 罗正, 李鸿博, 等. 微生态制剂对蛋鸡生长性能和肠道菌群及抗体效价的影响 [J]. 中国畜牧杂志, 2015, 51(5): 60-64.

[11] SANTOSO U, OHTANI S, TANAKA K, et al. Dried Bacillus subtilis culture reduced ammonia release poultry house[J]. Asian-Australasian Journal of Animal Sciences, 1999, 12(5): 806-809.

[12] KIM H J, WOO J S, KWON O S, et al. Effects of Bacillus subtilis supplementation on egg quality, blood characteristics and fecal NH3-N in laying hens[J]. Korean Journal of Poultry Science, 2005, 32(1): 9-14.

[13] ABDELQADER A, AL-FATAFTAH A R, DAŞ G. Effects of dietary Bacillus subtilis and inulin supplementation on performance, eggshell quality, intestinal morphology and microflora composition of laying hens in the late phase of production[J]. Animal Feed Science and Technology, 2013, 179(1/2/3/4): 103-111.

[14] GUO J R, DONG X F, LIU S, et al. Effects of long-term Bacillus subtilis CGMCC 1.921 supplementation on performance, egg quality, and fecal and cecal microbiota of laying hens[J]. Poultry Science, 2016, 96(5): 1280-1289.

[15] 王翔宇. 枯草芽孢杆菌对蛋鸡生产性能及蛋品质的影响 [D]. 硕士学位论文. 长春: 吉林大学, 2015.

[16] RIBEIRO V, Jr., ALBINO L F T, ROSTAGNO H S, et al. Effects of the dietary supplementation of Bacillus subtilis, levels on performance, egg quality

and excreta moisture of layers[J]. Animal Feed Science and Technology, 2014, 195: 142-146.

[17] SOBCZAK A, KOZŁOWSKI K. The effect of a probiotic preparation containing Bacillus subtilis ATCC PTA-6737 on egg production and physiological parameters of laying hens[J]. Annals of Animal Science, 2015, 15(3): 711-723.

[18] 丁海. 枯草芽孢杆菌对蛋鸡生产性能及盲肠菌群区系影响的研究 [D]. 硕士学位论文. 银川: 宁夏大学, 2013.

[19] 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.

[20] 翟茹环. 枯草芽孢杆菌 G8 抗菌物质的理化性质分析及分离纯化 [D]. 硕士学位论文. 泰安: 山东农业大学, 2008.

[21] KNARREBORG A, BROCKMANN E, HØYBYE K, et al. Bacillus subtilis (DSM17299) modulates the ileal microbial communities and improves growth performance in broilers[J]. International Journal of Probiotics & Prebiotics, 2008, 3(2): 83-88.

[22] 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.

[23] SAMANYA M, YAMAUCHI K E. Histological alterations of intestinal villi in chickens fed dried Bacillus subtilis var. natto[J]. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 2002, 133(1): 95-104.

[24] TEO A Y, TAN H M. Evaluation of the performance and intestinal gut microflora of broilers fed on corn-soy diets supplemented with Bacillus subtilis PB6 (CloSTAT)[J]. The Journal of Applied Poultry Research, 2007, 16(3): 296-303.

[25] 江国忠. 高产纤维素酶枯草芽孢杆菌的筛选、应用及其产酶条件研究 [D]. 硕士学位论文. 南昌: 南昌大学, 2010.

[26] SCHOLZ-AHRENS K E, ADE P, MARTEN B, et al. Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure[J]. The Journal of Nutrition, 2007, 137(3): 838S-846S.

[27] BAIN M M. Recent advances in the assessment of eggshell quality and their future application[J]. World' s Poultry Science Journal, 2005, 61(2): 268-277.

[28] AGRONOMIQUE F. Egg formation and chemistry[M]//NYS Y, BAIN M, VAN IMMERSEEL F. Improving the safety and quality of eggs and egg products: egg chemistry, production and consumption. Cambridge: Woodhead Publishing, 2011: 83.

[29] TSAI W T, YANG J M, LAI C W, et al. Characterization and adsorption properties of eggshells and eggshell membrane[J]. Bioresource Technology, 2006,

97(3): 488-493.

[30] MICHAEL B C. Involvement of calcium and phosphorus in bone and shell quality of early maturing commercial layers[J]. World Poultry, 2001, 6: 16-19.

[31] ABDELQADER A, IRSHAID R, AL-FATAFTAH A R. Effects of dietary probiotic inclusion on performance, eggshell quality, cecal microflora composition, and tibia traits of laying phase production[J]. Tropical animal health production, 2013, 45(4): 1017-1024.

[32] 裴跃明, 邵强, 吴桂龙, 等. 枯草芽孢杆菌制剂对产蛋后期蛋鸡生产性能、蛋品质、免疫及肠道菌群的影响 [J]. 中国畜牧杂志, 2016, 52(7): 61-65, 70.

[33] AL-BATSHAN H A, SCHEIDELER S E, BLACK B L, et al. Duodenal calcium uptake, femur ash, and eggshell quality decline with age and increase following molt[J]. Poultry Science, 1994, 73(10): 1590-1596.

[34] GROBAS S, MENDEZ J, DE BLAS C, et al. Influence of dietary energy, supplemental fat and linoleic acid concentration on performance of laying hens at two ages[J]. British Poultry Science, 1999, 40(5): 681-687.

[35] ASHMEAD H D, GRAFF D J, ASHMEAD H H. Factors affecting mineral absorption from the intestine[M]//CHARLES C. Intestinal absorption of metal ions. Springfield: Thomas Pub., 1985: 13-29.

[36] 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.

[37] ŚWIĄTKIEWICZ S, KORELESKI J, ARCZEWSKA A. Laying performance and eggshell quality in laying hens fed diets supplemented with prebiotics and organic acids[J]. Czech Journal of Animal Science, 2010, 55(7): 294-304.

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