Effects of Bacillus megaterium 1259 Preparation versus Yucca Extract and Bacillus subtilis on Production Performance and Ammonia Nitrogen Content in Excreta of Laying Hens (Postprint)

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Abstract

This study aimed to compare the effects of dietary supplementation with Bacillus megaterium 1259 (BM1259) preparation, yucca extract, and Bacillus subtilis on the production performance of laying hens and ammonia nitrogen content in excreta. A total of 384 healthy 300-day-old Hy-Line Brown laying hens with similar laying rates were selected and randomly divided into 4 groups, with 6 replicates per group and 16 hens per replicate. The control group was fed a basal diet, while the treatment groups were fed the basal diet supplemented with 100 mg/kg BM1259 preparation, yucca extract, and Bacillus subtilis, respectively. The pre-experimental period lasted 14 days, and the formal experimental period lasted 42 days. The results showed that: 1) Compared with the control group, the BM1259 group extremely significantly increased total egg weight, egg number, laying rate, and average egg weight ($P<0.01$), and extremely significantly decreased feed-to-egg ratio ($P<0.01$). These results were similar to those of the Bacillus subtilis group and superior to the yucca extract group. 2) Compared with the control group, the BM1259 group extremely significantly decreased ammonia nitrogen, uric acid, urea nitrogen contents, and urease activity in laying hen excreta ($P<0.01$). These results were similar to those of the yucca extract group and superior to the Bacillus subtilis group. 3) Dietary supplementation with BM1259 preparation could achieve greater economic benefits compared with supplementation with yucca extract or Bacillus subtilis. In conclusion, dietary supplementation with BM1259 preparation in laying hens can improve production performance, increase profits, and possess superior ammonia and odor removal effects.

Full Text

Effects of Bacillus megaterium 1259 Preparation Compared with Yucca and Bacillus subtilis on Production Performance and Ammonia Nitrogen Content in Excrement of Laying Hens

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Abstract: This study was conducted to compare the effects of dietary supplementation with Bacillus megaterium 1259 (BM1259) preparation versus yucca extract and Bacillus subtilis on production performance and ammonia nitrogen content in the excrement of laying hens. A total of 384 healthy 300-day-old Hy-Line Brown laying hens with similar laying rates were randomly allocated into four groups, each consisting of six replicates of 16 hens. The control group received a basal diet, while the experimental groups were fed the basal diet supplemented with 100 mg/kg BM1259 preparation, yucca extract, or Bacillus subtilis, respectively. The study included a 14-day preliminary period followed by a 42-day formal experimental period. The results demonstrated that: (1) Compared with the control group, BM1259 supplementation significantly increased total egg mass, egg production, laying rate, and average egg weight ($P < 0.01$), while significantly decreasing the feed-to-egg ratio ($P < 0.01$). These effects were comparable to those observed with Bacillus subtilis and superior to those of yucca extract. (2) BM1259 significantly reduced ammonia nitrogen, uric acid, urea nitrogen content, and urease activity in excrement ($P < 0.01$), with efficacy similar to yucca extract and better than Bacillus subtilis. (3) Dietary inclusion of BM1259 preparation yielded greater economic benefits compared with yucca or Bacillus subtilis supplementation. In conclusion, BM1259 preparation improved production performance, increased profitability, and exhibited superior ammonia and odor removal effects in laying hens.

Keywords: *Bacillus megaterium*; yucca extract; *Bacillus subtilis*; laying hens; production performance; ammonia nitrogen content

Introduction

Ammonia volatilization and the resulting malodor have long been significant concerns in poultry production, with ammonia emissions from livestock operations representing a major global pollutant [1]. While conventional ventilation methods can reduce ammonia concentrations within poultry houses, they merely

transfer the pollution to the external environment. Among deodorizing feed additives, only yucca extract is currently listed in China' s latest "Feed Additive Catalogue (2013)" [2]; however, its high cultivation requirements, reliance on imports, limited availability, and high cost have restricted its widespread application. *Bacillus subtilis* (BS) is an approved probiotic in China and represents the most extensively used *Bacillus* additive both domestically and internationally, with numerous studies investigating its application in chicken production. *Bacillus megaterium* 1259 (BM1259) preparation is a novel deodorizing feed additive under development that has demonstrated promising deodorizing effects and production-enhancing properties. Previous research by Su et al. [3] found that dietary yucca extract supplementation significantly reduced ammonia nitrogen content, while Wang et al. [4] reported that *Bacillus subtilis* addition in broiler diets decreased ammonia emissions from fermented manure. Our research group previously demonstrated that dietary supplementation with 2$×10^{6}$ CFU/g BM1259 reduced air ammonia content by 53–74% and hydrogen sulfide by 55–90% in closed poultry houses during winter [5], indicating its potential as a deodorizing feed additive. Furthermore, dose-response studies in laying hens showed that BM1259 improved production performance with a positive correlation between dosage and deodorizing efficacy, with 100 mg/kg being the optimal level. However, comparative studies evaluating BM1259 against other types of deodorizing additives remain scarce. Therefore, this experiment was designed to compare BM1259 with yucca extract and *Bacillus subtilis*—two well-studied additives—by evaluating their effects on laying hen performance and fecal ammonia nitrogen content, thereby providing a basis for BM1259 application in improving both production performance and environmental welfare by reducing volatile ammonia nitrogen hazards.

1.1 Experimental Design

A total of 384 healthy 300-day-old Hy-Line Brown laying hens with uniform body weight and laying rate ($P > 0.05$) were randomly divided into four groups, each comprising six replicates of 16 hens. The control group received a basal diet formulated according to Hy-Line Brown nutrient requirements (composition and nutrient levels shown in Table 1), while the three experimental groups received the basal diet supplemented with 100 mg/kg BM1259 preparation, yucca extract, or *Bacillus subtilis*, respectively. The study consisted of a 14-day preliminary period followed by a 42-day formal experimental period.

1.2 Experimental Materials

The BM1259 preparation was provided by the research group "Development of Novel Biological Feed Additive BM1259" (Batch No.: 20120731), containing viable *Bacillus megaterium* at 1$×10^{10}$ CFU/g. Yucca extract was purchased from Shanghai Youjiu Biological Technology Co., Ltd. (Batch No.: BPW-080) with a recommended dosage of 100 mg/kg. *Bacillus subtilis* was obtained from Yangzhou Lvke Biological Co., Ltd., also with a recommended dosage of 100

mg/kg.

1.3 Management Practices

The experimental poultry house utilized natural ventilation with two hens per cage. Birds had ad libitum access to feed and water under a lighting regimen of 16.5 hours light and 7.5 hours darkness. Manure was removed regularly to maintain house hygiene.

1.4 Excrement Collection

Fresh excrement samples uncontaminated within 30 minutes of defecation were collected from each replicate at 08:00 on days 28, 31, and 35 of the formal experimental period. Samples were immediately placed in ice boxes, sealed, and stored at -40°C.

1.5 Production Performance Metrics

During the experimental period, daily egg number, egg weight, feed intake, and mortality were recorded by replicate. Total egg mass, egg production, laying rate, average egg weight, average daily feed intake, feed-to-egg ratio, and survival rate were calculated according to the "Chinese Agricultural Industry Standard for Poultry Production Performance Terminology and Statistical Methods" [6].

1.6 Determination of Ammonia Nitrogen Content and Urease/Uricase Activity in Excrement

Ammonia nitrogen content was determined using the indophenol blue colorimetric method [7]. Urea nitrogen and uric acid contents were measured using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute. Urease activity was also assessed via the indophenol blue colorimetric method [7]. Uricase activity was determined by measuring the change in optical density at 280 nm, where uric acid exhibits an absorption peak [8]; activity was expressed as the difference in optical density before and after reaction, with relative activity calculated using the control group as baseline.

1.7 Statistical Analysis

Data were organized using Excel and analyzed with SPSS 18.0 software via one-way ANOVA. Multiple comparisons were performed using the LSD method, with significance set at $P < 0.05$ and $P < 0.01$.

Results

2.1 Effects of BM1259, Yucca, and *Bacillus subtilis* on Production Performance

As shown in Table 2 , compared with the control group, both BM1259 and *Bacillus subtilis* significantly increased total egg mass and average egg weight ($P < 0.01$). BM1259 and yucca significantly reduced the feed-to-egg ratio ($P < 0.01$). BM1259 significantly improved egg production and laying rate ($P < 0.01$), while *Bacillus subtilis* produced significant improvements in these parameters ($P < 0.05$). In contrast, yucca significantly decreased laying rate and daily feed intake per hen ($P < 0.05$) and markedly reduced survival rate ($P < 0.01$).

When comparing treatment groups, BM1259 showed significantly superior performance to yucca in total egg mass, egg production, laying rate, and survival rate ($P < 0.01$), and significantly higher daily feed intake ($P < 0.05$). Compared with *Bacillus subtilis*, BM1259 significantly improved the feed-to-egg ratio ($P < 0.01$).

2.2 Effects on Ammonia Nitrogen, Urea Nitrogen, and Uric Acid Content in Excrement

Table 3 reveals that BM1259 and yucca significantly reduced ammonia nitrogen, urea nitrogen, and uric acid contents compared with the control group ($P < 0.01$), while *Bacillus subtilis* only significantly decreased urea nitrogen content ($P < 0.05$). Both BM1259 and yucca were significantly more effective than *Bacillus subtilis* in reducing urea nitrogen ($P < 0.01$) and uric acid ($P < 0.05$). No significant differences were observed between BM1259 and yucca groups except for ammonia nitrogen content, where BM1259 showed a significant advantage ($P < 0.05$).

2.3 Effects on Urease and Uricase Activity in Excrement

As presented in Table 4 , BM1259, yucca, and *Bacillus subtilis* all significantly reduced urease activity compared with the control group ($P < 0.01$). Although no significant differences in uricase activity were detected among groups ($P > 0.05$), all treatment groups exhibited lower uricase activity than the control. No significant differences in either enzyme activity were observed among the three treatment groups ($P > 0.05$).

2.4 Economic Analysis

A straightforward economic analysis was conducted to evaluate the profitability of the three additives. As shown in Table 5 , the basal diet cost 2.040 RMB/kg. The market reference price was 150 RMB/kg for yucca extract and 50 RMB/kg for both *Bacillus subtilis* and BM1259 preparations. Consequently, the final feed prices were 2.045 RMB/kg for BM1259 and *Bacillus subtilis* groups and 2.055 RMB/kg for the yucca group. Based on total feed costs and egg income, the

profits per hen were 6.280, 7.989, 7.231, and 7.109 RMB for the control, BM1259, yucca, and *Bacillus subtilis* groups, respectively. The feed cost per kilogram of eggs produced was 5.059, 4.562, 4.683, and 4.835 RMB/kg, respectively. All three additives improved profitability and reduced egg production costs, with BM1259 demonstrating the highest profit and lowest feed cost per kilogram of eggs.

Discussion

Both *Bacillus megaterium* and *Bacillus subtilis* are *Bacillus* preparations that enhance production performance and improve the rearing environment in chicken production [9]. Yucca extract contains active components including steroidal saponins, polysaccharides, resveratrol, and polyphenols, which reduce ammonia emissions, modulate intestinal microenvironment, inhibit pathogens, and enhance immune function [10]. *Bacillus* species improve feed conversion and production performance through metabolic activity, synthesis of digestive enzymes, and modulation of intestinal flora, while significantly reducing ammonia nitrogen emissions. Anas et al. [11] reported that dietary supplementation with 1 g/kg *Bacillus subtilis* (2.3$×10^{5}$ CFU/g) significantly increased intestinal villus height and crypt depth in laying hens, thereby expanding the nutrient absorption area and improving feed conversion efficiency and egg production. Molnár et al. [12] found that *Bacillus subtilis* supplementation significantly improved broiler weight gain and feed conversion ratio. Wang et al. [13] demonstrated that *Bacillus subtilis* effectively reduced ammonia concentrations in broiler houses and significantly decreased total nitrogen, urea nitrogen, and serum ammonia nitrogen contents in excrement while improving nitrogen utilization. In the current study, *Bacillus subtilis* significantly increased total egg mass and average egg weight and improved egg production and laying rate, consistent with previous research. Additionally, *Bacillus subtilis* significantly reduced urease activity and ammonia nitrogen content in excrement, with a tendency to decrease uric acid content and uricase activity, indicating certain deodorizing capabilities. BM1259 showed no significant differences from *Bacillus subtilis* in production performance, with both improving economic returns, but demonstrated superior deodorizing efficacy by significantly reducing urea nitrogen and uric acid contents. Overall, BM1259 matches *Bacillus subtilis* in production performance while offering superior deodorizing effects.

Yucca extract functions as a urease inhibitor, with its macromolecules and sequential molecules adsorbing ammonia gas, while its polyphenol components strongly bind ammonia molecules. Cheng et al. [14] reported that dietary yucca supplementation significantly reduced ammonia concentrations in poultry houses, and Ma et al. [15] found it extremely effective in lowering ammonia levels. Yucca also improves intestinal microenvironment, enhances nutrient absorption, and boosts immune function. Jia et al. [16] observed that 60 mg/kg yucca significantly improved laying rate in broilers, while 120 mg/kg reduced the feed-to-egg ratio, demonstrating dose-dependent effects on production per-

formance. In this study, yucca significantly reduced ammonia nitrogen, urea nitrogen, uric acid contents, and urease activity in excrement, while substantially decreasing uricase activity. Based on ammonia nitrogen release patterns during excrement storage [17], these differences would likely manifest as even more significant reductions in atmospheric ammonia concentrations, indicating excellent deodorizing potential. BM1259 achieved deodorizing effects comparable to yucca, though our previous research [18] suggests the current dosage may not have fully realized BM1259' s deodorizing potential, leaving room for optimization. Regarding production performance, yucca only significantly improved the feed-to-egg ratio, consistent with Jia et al. [16]. Although economic benefits were similar between yucca and BM1259, BM1259 demonstrated superior overall production performance. Considering previous dose-response studies, the yucca dosage used here may have exceeded the optimal level for production performance enhancement, making the results consistent with expectations. These findings indicate that BM1259 offers comparable deodorizing efficacy to yucca while providing superior production performance improvements.

This comparative study demonstrates that BM1259 preparation provides comprehensive benefits, combining effective deodorizing properties with production performance enhancement—matching yucca' s deodorizing capabilities while rivaling *Bacillus subtilis* in production performance. Furthermore, BM1259 exhibited the best economic returns in this trial, providing a solid basis for its practical application in poultry production.

Conclusions

1. Dietary supplementation with BM1259 preparation increased total egg mass and average egg weight, significantly improved egg production and laying rate, and reduced the feed-to-egg ratio, with performance comparable to *Bacillus subtilis* and superior to yucca extract.
2. BM1259 supplementation decreased ammonia nitrogen content and uricase activity, and significantly reduced urea nitrogen, uric acid contents, and urease activity, with efficacy similar to yucca extract and slightly better than *Bacillus subtilis*.
3. Compared with yucca and *Bacillus subtilis*, dietary BM1259 supplementation generated the greatest economic benefits in laying hen production.

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