Research Note: Effects of *Bacillus coagulans* X26 on the production performance, intestinal structure, short-chain fatty acids and flora composition of laying hens during the peak laying period

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ABSTRACT This study investigated the effects of *Bacillus coagulans* X26 on the production performance, egg quality, intestinal structure, intestinal short-chain fatty acids, and microbial diversity of laying hens during the peak laying period and explored the possibility of using *B. coagulans* X26 as an alternative to antibiotics. The results showed that adding 1.0×10^6 CFU/g *B. coagulans* X26 to the basal diet had the best effect: the average laying rate increased by 4.20% ($P < 0.05$), the survival rate increased by 7.15% ($P < 0.05$), the feed intake decreased by 3.06% ($P < 0.05$), the ratio of feed to egg decreased by 7.42% ($P < 0.05$), the rate of soft-broken eggshell decreased by 73.04% ($P < 0.05$), the average egg weight increased by 2.94% ($P < 0.05$), and the content of egg white protein increased by 5.77% ($P < 0.05$). The production performance and egg quality of laying hens reached the same level as that of hens fed a diet supplemented with chlortetracycline hydrochloride in this study, and there were significant advantages in the average laying rate and feed-egg ratio ($P < 0.05$). Both chlortetracycline hydrochloride and *B. coagulans* X26 altered the flora composition and the SCFA content of the intestinal contents; however, *B. coagulans* X26 also significantly increased the villus height of the ileum and the ratio of villus height to crypt depth ($P < 0.05$).

Key words: *B. coagulans* X26, production performance, SCFA, intestinal structure, flora composition

2022 Poultry Science 101:101835
https://doi.org/10.1016/j.psj.2022.101835

INTRODUCTION

Antibiotic abuse has had severe effects on human health, and probiotics have been widely used in the animal husbandry industry as an alternative to antibiotics. *Bacillus coagulans* belongs to a class of probiotics with high resistance. The spore body of this species is resistant to gastric acid, bile salts, high temperature and high pressure, allowing it to adapt to the animal gastrointestinal environment and to meet the requirements for the processing of granule feed to form a stable high-quality product. Cavazzoni et al. (1998) found that the use of *B. coagulans* in broilers had effects on growth and disease prevention comparable to those of Virgamycin. Studies by Zhen et al. (2018) showed that the addition of *B. coagulans* to broiler diets improved the growth performance, intestinal microbiota, and villus structure of broilers and repaired the intestinal inflammation and structural damage caused by *Salmonella* enteritis infection. Chunru Liu et al. (2022) found that *B. coagulans* could maintain the intestinal mucosal barrier by improving intestinal flora, enhancing innate immunity, and promoting intestinal epithelial proliferation. In this study, the effect of *B. coagulans* X26 on production performance, egg quality, gut structure, and intestinal flora was analyzed in the peak period of egg production; in addition, the results were compared with those of chlortetracycline hydrochloride to provide an effective reference for the rational application of *B. coagulans* X26 to improve laying production and to explore its mechanism of action.

MATERIALS AND METHODS

Experimental Design

Based on a univariate experimental design, 160 healthy Hailan laying hens with similar egg yields at 42 wk and insignificant weight differences were selected and randomly divided into 4 treatment groups (5 replicates for each group, 8 hens per replicate): control group, basic diet; antibiotic group, basic diet supplemented with 0.1 mg/g chlortetracycline hydrochloride; test

Received November 26, 2021.
Accepted February 25, 2022.

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group 1, basic diet supplemented with 5.0×10^5 CFU/g *B. coagulans* X26; and test group 2, basic diet supplemented with 1.0×10^6 CFU/g *B. coagulans* X26. The rearing trial lasted for 5 wk.

The strain was deposited in the China Center for Type Culture Collection (CCTCC M 20211707). The composition of the basic diet was previously described in NRC (1994).

Determination of Indices

High-performance liquid chromatography was used to detect SCFAs in intestinal contents. Using Image-Pro Plus 6.0 software with the 40-fold scale in the lower right corner as the standard, 5 intact villi were selected from each section, and the villus height (mm) and the crypt depth (mm) were measured. The V3-V4 region of the 16S rRNA gene of the cecal microbiota was sequenced using 454 high-throughput sequencing for specific analysis. All raw sequences were deposited in the NCBI Sequence Read Archive under accession number PRJNA672222.

Statistical Analysis

Data are presented as the mean and standard deviation (**SD**) and were analyzed by ANOVA using SPSS 16.0 for multiple comparisons with Duncan's test. Two independent sample t tests were used to compare independent data between 2 groups, and F tests were used to compare data between multiple groups. $P < 0.05$ indicates a significant difference.

RESULTS AND DISCUSSION

Effect of B. coagulans *X26 on the Production Performance and Egg Quality of Laying Hens*

The data of this study (Table 1) showed that compared with the control group, the survival rate of laying hens increased by 5.36% ($P < 0.05$), and the soft shelled or cracked egg rate decreased by 53.91% ($P < 0.05$) in the antibiotic group. The survival rate of laying hens increased by 5.36% ($P < 0.05$), and the soft-shelled or cracked egg rate decreased by 81.74% ($P < 0.05$) in test group 1. Test group 2 showed significantly improved production performance. The average laying rate increased by 4.20% ($P < 0.05$); the average egg weight increased by 2.94%; the survival rate of laying hens increased by 7.15% ($P < 0.05$); the ADFI decreased by 3.06% ($P < 0.05$); the feed-egg ratio decreased by 7.42% ($P < 0.05$); and the soft-shelled or cracked egg rate decreased by 73.04% ($P < 0.05$). Compared with the antibiotic group, the soft shelled or cracked egg rate was reduced by 60.38% ($P < 0.05$) in test group 1; the average laying rate in test group 2 increased by 2.69% ($P < 0.05$), and the feed-egg ratio was decreased by 4.93% ($P < 0.05$) in test group 2. The results showed that the addition of chlortetracycline hydrochloride and *B. coagulans* X26 to the basic diet can improve the performance of laying hens. *B. coagulans* X26 can match the effect of antibiotics on the production performance of laying hens and even exhibits advantages in terms of laying rate and feed-egg ratio. Compared with test group 1, the average laying rate of test group 2 was significantly increased ($P < 0.05$). This shows that different bacterial counts of *B. coagulans* X26 have different degrees of influence on the production performance of laying hens.

Compared with that of the control group, the egg yolk protein content of the antibiotic group increased by 3.86% ($P < 0.05$), the egg white protein content of test group 1 increased by 8.60% ($P < 0.05$), the average egg weight of test group 2 increased by 2.94%, and the egg white protein content of test group 2 increased by 5.77% ($P < 0.05$). Compared with the antibiotic group, test groups 1 and 2 showed similar values for 9 egg quality indices, which indicated that *B. coagulans* X26 had the same effect on egg quality as antibiotics. The results showed that adding *B. coagulans* X26 to the basic diet could improve the quality of eggs.

Effect of B. coagulans *X26 on Intestinal Structure*

The process of digestion and absorption of nutrients occurs mainly in the small intestine, and the villi of the

Table 1. Production performance and egg quality.

Index	Control group	Antibiotic group	Test group 1	Test group 2
Average laying rate, %	89.43 ± 2.53^{a}	90.75 ± 2.51^{a}	92.04 ± 2.97^{a}	93.19 ± 1.17^{b}
ADFI, g	127.49 ± 3.87^{b}	125.56 ± 3.34^{ab}	124.97 ± 2.53^{ab}	123.59 ± 1.98^{a}
Average egg weight, g	61.54 ± 0.64^{a}	61.96 ± 1.42^{ab}	61.98 ± 1.18^{ab}	63.35 ± 1.12^{b}
Feed-egg ratio	2.29 ± 0.04^{b}	2.23 ± 0.11^{b}	2.19 ± 0.05^{ab}	2.12 ± 0.05^{a}
Soft shelled or cracked egg rate, %	1.15 ± 0.23^{c}	0.53 ± 0.18^{b}	0.21 ± 0.22^{a}	0.31 ± 0.11^{ab}
Survival rate of laying hens, %	93.33 ± 3.73^{a}	98.33 ± 3.73b	98.33 ± 3.73^{b}	100.00 ± 0.00^{b}
Egg shape index	1.32 ± 0.06	1.40 ± 0.99	1.31 ± 0.06	1.32 ± 0.04
Relatively heavy egg yolk, %	27.84 ± 1.15	28.62 ± 1.29	28.26 ± 1.30	27.27 ± 1.37
Relatively heavy eggshell, %	10.31 ± 0.21	10.23 ± 0.34	10.44 ± 0.41	10.50 ± 0.52
Egg white water content, %	87.46 ± 0.40	87.47 ± 0.31	86.89 ± 0.55	87.25 ± 0.45
Egg yolk moisture content, %	45.81 ± 0.35	44.71 ± 0.92	45.96 ± 0.23	45.49 ± 0.70
Fat content (fresh eggs), %	8.08 ± 0.37	8.49 ± 0.46	8.27 ± 0.47	8.08 ± 0.49
Egg white protein content, %	9.54 ± 0.32^{a}	9.86 ± 0.26^{ab}	10.36 ± 0.58^{b}	10.09 ± 0.39^{b}
Egg yolk protein content, %	16.85 ± 0.18^{a}	17.50 ± 0.40^{b}	16.96 ± 0.38^{ab}	16.95 ± 0.51^{ab}

In the same row, values with the same or no letter superscripts mean no significant difference ($P > 0.05$), whereas with different small letter superscripts mean significant difference ($P < 0.05$).

Table 2. Intestinal content analysis.

Intestinal tract	Index	Control group	Antibiotic group	Test group 1	Test group 2
Duodenum, mm	Villus Height	1.15 ± 0.02	1.12 ± 0.22	1.38 ± 0.25	1.22 ± 0.25
	Crypt depth	0.13 ± 0.03	0.14 ± 0.01	0.16 ± 0.03	0.14 ± 0.01
	V/C	8.85 ± 0.11	8.00 ± 0.38	8.63 ± 0.51	8.71 ± 0.45
Jejunum, mm	Villus Height	0.92 ± 0.16	0.69 ± 0.30	0.81 ± 0.19	0.82 ± 0.21
	Crypt depth	0.14 ± 0.01	0.17 ± 0.05	0.14 ± 0.04	0.14 ± 0.04
	V/C	6.57 ± 0.26	4.06 ± 0.56	5.79 ± 0.33	5.86 ± 0.45
Ileum, mm	Villus Height	0.57 ± 0.11^{a}	0.54 ± 0.25^{a}	0.89 ± 0.09^{b}	0.80 ± 0.22^{b}
	Crypt depth	0.11 ± 0.02	0.11 ± 0.03	0.12 ± 0.02	0.11 ± 0.02
	V/C	5.18 ± 0.14^{a}	4.91 ± 0.25^{a}	7.42 ± 0.25^{b}	7.27 ± 0.25^{b}
Ileum, mg/g	Formic acid	1.98 ± 0.42^{ab}	1.09 ± 0.29^{a}	2.49 ± 0.70^{bc}	3.15 ± 0.69^{c}
	Acetic acid	0.55 ± 0.09^{c}	1.31 ± 0.45^{d}	0.47 ± 0.10^{bc}	0.28 ± 0.05^{ab}
	Butanoic acid	0.04 ± 0.01^{a}	0.12 ± 0.03^{c}	0.08 ± 0.02^{b}	0.10 ± 0.03^{bc}
	Lactic acid	1.94 ± 0.09^{a}	2.52 ± 0.15^{a}	4.00 ± 0.67^{b}	4.11 ± 0.84^{b}
	Subtotal	4.46 ± 0.48^{a}	5.04 ± 0.47^{a}	7.04 ± 0.71^{b}	7.64 ± 0.90^{b}
Cecum, mg/g	Formic acid	0.01 ± 0.01^{a}	0.62 ± 0.23^{c}	0.07 ± 0.01^{b}	0.19 ± 0.06^{b}
	Acetic acid	4.18 ± 0.50^{b}	4.44 ± 0.85^{b}	2.26 ± 0.62^{a}	2.02 ± 0.42^{a}
	Butanoic acid	0.02 ± 0.01^{a}	0.36 ± 0.12^{b}	0.21 ± 0.09^{b}	0.74 ± 0.24^{c}
	Lactic acid	23.96 ± 1.30	29.09 ± 3.38	27.34 ± 4.32	25.19 ± 6.16
	Subtotal	28.14 ± 2.32	34.51 ± 4.30	29.88 ± 3.01	28.14 ± 1.35

In the same row, values with the same or no letter superscripts mean no significant difference ($P > 0.05$), whereas with different small letter superscripts mean significant difference ($P < 0.05$).

small intestine extend into the intestinal cavity, which is the first barrier for the digestion and absorption of nutrients. The villus height, crypt depth, and ratio of villus height to crypt depth all reflect the strength of intestinal digestion and absorption ability. Longer villi, a shallower crypt and a higher ratio of villus height to crypt depth are indicative of more mature and perfect intestinal mucosal cells and of a stronger intestinal digestion and absorption ability (Paiva et al., 2014). In this study, Table 2 shows that there was no significant difference in the villus height or crypt depth between the duodenum and jejunum. However, the villus height and the ratio of villus height to crypt depth of the ileum in test groups 1 and 2 were significantly superior. Compared with the control group, villus height increased by 56.14% ($P < 0.05$), and the ratio of villi height to crypt depth increased by 43.24% ($P < 0.05$) in test group 1; the villus height in test group 2 increased by 40.35% ($P < 0.05$), and the ratio of villus height to crypt depth increased by 40.34% ($P < 0.05$). Compared with the antibiotic group, villus height increased by 64.81% ($P < 0.05$), and the ratio of villi height to crypt depth increased by 51.12% ($P < 0.05$) in test group 1; the villus height in test group 2 increased by 48.15% ($P < 0.05$), and the ratio of villus height to crypt depth increased by 48.07% ($P < 0.05$). The results showed that *B. coagulans* X26 significantly increased the ileal villus height and the ratio of villus height to crypt depth ($P < 0.05$) as an additive to the basic diet, whereas chlortetracycline hydrochloride had no significant effect.

Effect of B. coagulans *X26 on SCFAs in Intestinal Content*

Camille et al. (2021) reviewed the importance of the mechanism and function of SCFAs in the gut: SCFAs are metabolites of bacterial fermentation of dietary fiber, which links host nutrition to the maintenance of intestinal homeostasis. SCFAs are important energy donors for intestinal epithelial cells and regulate the proliferation and differentiation of these cells, as well as the functions of subsets of enteroendocrine cells through different mechanisms. They affect intestinal motility and enhance intestinal barrier function and host metabolism. The results of this study (Table 2) showed that chlortetracycline hydrochloride increases the levels of acetic acid and butyric acid in the ileum and those of formic acid and butyric acid in the cecum, and *B. coagulans* X26 increases the levels of formic acid, butyric acid, lactic acid, and total acid in the ileum and the levels of formic acid and butyric acid in the cecum. The addition of chlortetracycline hydrochloride and *B. coagulans* X26 to the basal diet had different effects on intestinal SCFAs; however, the content of butyric acid in the ileum and cecum in each group increased significantly, especially in test group 2. The content of butyric acid was the highest, but *B. coagulans* X26 itself did not produce butyric acid, so it increased the content of butyric acid through a different mechanism. However, the decrease in acetic acid content in test group 1 and test group 2 may be attributed to active metabolism in the liver, as most of the acetic acid in the intestinal tract is transported to the liver for metabolism and energy supply; alternatively, it may be converted to butyric acid for utilization. Test group 1 and test group 2 both showed a significantly increased level of lactic acid in the ileum, which was consistent with the ability of *B. coagulans* X26 to produce lactic acid.

Effect of B. coagulans *X26 on Intestinal Microbes in Laying Hens*

In this study, the results of microbial high-throughput sequencing and microflora diversity analysis showed

that the dominant genera in the cecum of laying hens were *Bacteroides, Lactobacillus, Phascolarctobacterium, Oscillospira, Prevotella, Faecalibacterium, Subdoligranulum, Ruminococcus, Ruminococcaceae_Ruminococcus*, and *Parabacteroides*, and the abundances varied among the four test groups. Low-dose *B. coagulans* X26 increased the abundance of *Phascolarctobacterium* in the cecum and decreased the abundance of *Bacteroides, Prevotella, Faecalibacterium* and *Ruminococcus* in the cecum. In addition, high-dose *B. coagulans* X26 also increased the abundance of *Lactobacillus* and decreased the abundance of *Oscillospira*. Different numbers of *B. coagulans* X26 have different effects on intestinal flora.

The ratio of the abundances of *Bacteroides* to *Lactobacillus* in the test groups were close to those of the antibiotic group. Also, the effect of *B. coagulans* X26 in improving the performance of laying hens was similar to that of chlortetracycline hydrochloride. *Bacteroides* can be pathogenic when the barrier function of organisms is damaged, such as when the flora is out of balance, the resistance of organisms is reduced, the blood supply of local tissues is obstructed, etc. However, there are few reports about the pathogenicity of *Lactobacillus*. The results indicated that the abundance ratio of *Bacteroides* to *Lactobacillus* might be related to the performance of laying hens.

In summary, within the scope of this study, adding a high dose of *B. coagulans* X26 (1.0×10^6 CFU/g) to the basic diet during the peak laying period of laying hens had the best effect, with egg quality and laying performance reaching the same levels as those of antibiotics, and *B. coagulans* X26 showed significant advantages in terms of average laying rate and feed-egg ratio ($P < 0.05$). The production performance of the low-dose test group did not reach the same level as that of the high-dose test group, which indicated that the effect of *B. coagulans* X26 was closely related to the amount of *B. coagulans* X26 in the diet. It is speculated that *B. coagulans* X26 can improve the nutrient digestion and absorption ability of laying hens by improving the composition and structure of intestinal flora, increasing the content of SCFAs in the intestine and improving intestinal health to improve production performance and egg quality. The role of probiotics in different fields has been widely reported, but research on the mechanism underlying the effect of *B. coagulans* as a probiotic is not comprehensive, and systematic in-depth research is needed.

ACKNOWLEDGMENTS

The author thanks all the people who participated in this project. We would like to thank Elsevier (https://webshop.elsevier.com/) for English language editing.

DISCLOSURES

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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