Effects of Probiotic Preparation on Growth Performance and Intestinal Health in Stressed Male Broiler Chickens: Postprint

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Abstract

This experiment aimed to investigate the effects of stress on growth performance and intestinal health in male broiler chickens, as well as the potential alleviating effect of probiotics. The study utilized a stress model of glucocorticoid (dexamethasone) injection in male broiler chickens. A total of 176 one-day-old Arbor Acres (AA) male broiler chickens were selected and arranged in a 2$×$2 factorial design, divided into four groups: stress+probiotics group (subcutaneous injection of 2 mg/kg BW dexamethasone, drinking water supplemented with probiotics at 0.02 g/bird/day), stress group (injection of 2 mg/kg BW dexamethasone), probiotics group (injection of 2 mg/kg BW saline, drinking water supplemented with probiotics at 0.02 g/bird/day), and control group (injection of 2 mg/kg BW saline). Each group had 4 replicates with 11 birds per replicate. The probiotics group and stress+probiotics group received probiotics dissolved in cooled boiled water via drinking water during days 11-14 and 25-28, while the stress group and control group received the same volume of cooled boiled water without probiotics. The stress group and stress+probiotics group were subcutaneously injected with dexamethasone sodium phosphate on days 12-14 and 26-28, while the probiotics group and control group were injected with the same dose of saline. The experimental period lasted 35 days. The results showed that stress significantly decreased ($P<0.05$) the average body weight, average daily gain, and European Performance Index at 35 days of age, significantly increased ($P<0.05$) the feed conversion ratio and mortality and culling rate, and significantly reduced ($P<0.05$) the immune organ index and jejunal villus height at 15 and 29 days of age in male broiler chickens; probiotics significantly increased ($P<0.05$) pancreatic lipase activity and jejunal villus height, and significantly decreased ($P<0.05$) crypt depth at 29 days of age in male broiler chickens. A significant interaction ($P<0.05$) between stress and probiotics was observed for

the villus height to crypt depth ratio. The results suggest that stress can disrupt the intestinal microenvironment, reduce growth performance, and inhibit normal development of immune organs in male broiler chickens; probiotics can significantly alleviate stress-induced damage to intestinal villi, but no significant effect of probiotics on growth performance was observed.

Full Text

Effects of Probiotics on Growth Performance and Intestinal Health of Stressed Male Broilers

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Abstract

This experiment investigated the effects of stress on growth performance and intestinal health in male broilers and the potential ameliorative role of probiotics. A stress model was established by injecting broilers with glucocorticoid (dexamethasone). One hundred seventy-six 1-day-old Arbor Acres (AA) male broilers were selected and allocated to four groups according to a 2$×$2 factorial design: stress + probiotics group (subcutaneous injection of 2 mg/kg BW dexamethasone and drinking water supplementation with 0.02 g probiotics per bird per day), stress group (injection of 2 mg/kg BW dexamethasone), probiotics group (injection of 2 mg/kg BW saline and drinking water supplementation with 0.02 g probiotics per bird per day), and control group (injection of 2 mg/kg BW saline). Each group comprised four replicates with 11 broilers per replicate. The probiotics group and stress + probiotics group received probiotics dissolved in cooled boiled water via drinking water during days 11–14 and 25–28, while the stress group and control group received cooled boiled water without probiotics. The stress group and stress + probiotics group were subcutaneously injected with dexamethasone sodium phosphate during days 12–14 and 26–28, whereas the probiotics group and control group received the same dose of saline. The experiment lasted 35 days. Results showed that stress significantly reduced average body weight, average daily gain, and European Performance Index at 35 days of age ($P<0.05$), while significantly increasing feed-to-gain ratio and mortality rate ($P<0.05$). Stress also significantly decreased immune organ indices and jejunal villus height at 15 and 29 days of age ($P<0.05$). Probiotics significantly increased pancreatic lipase activity and jejunal villus height at 29 days of age ($P<0.05$) while significantly reducing crypt depth ($P<0.05$). A significant interaction between stress and probiotics was observed for the villus height-to-crypt

depth ratio (P<0.05). These findings indicate that stress disrupts the intestinal microenvironment, reduces growth performance, and inhibits normal immune organ development in male broilers, while probiotics can significantly alleviate stress-induced damage to intestinal villi, though no significant effects on growth performance were observed.

Keywords: probiotics; male broilers; stress; growth performance; jejunum morphology; enzyme activity

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Poultry production environments involve multiple stressors that induce glucocorticoid secretion, causing detrimental effects on production performance [1]. Probiotics are live microbial preparations that can regulate microecological balance by supplementing normal intestinal flora, thereby excluding and inhibiting aerobic pathogen growth and modulating the intestinal environment [2]. Previous research by He et al. [3] demonstrated that stress disrupts internal homeostasis, prolongs the feeding period of broilers, and severely impacts the broiler industry. Studies by Hu et al. [4] and Cheng et al. [5] showed that probiotics can improve the intestinal environment of broilers, significantly increase market weight, and reduce feed-to-gain ratio, while also improving egg production, fertility, and hatchability in breeding hens while reducing feed-to-egg ratio and broken egg rate [6]. However, few reports have examined the effects of probiotics on broilers under stress conditions. Therefore, this study aimed to investigate the impacts of stress on growth performance and intestinal health in male broilers and the ameliorative effects of probiotics, providing theoretical basis and scientific methods for rational probiotic application in large-scale broiler production.

1. Materials and Methods

The probiotic preparation was provided by Jiangsu Hengfengqiang Biotechnology Co., Ltd., containing viable bacteria counts of: Bifidobacterium $ 10^{7}$ CFU/g, Lactobacillus $ 10^{7}$ CFU/g, Streptococcus faecalis $ 10^{4}$ CFU/g, and yeast $ 10^{6}$ CFU/g. Dexamethasone sodium phosphate injection (active ingredient: dexamethasone) was purchased from Chenxin Pharmaceutical Co., Ltd.

1.2 Experimental Design

One hundred seventy-six 1-day-old Arbor Acres (AA) male broilers with similar body weights were selected and randomly divided into four groups using a 2$×$2 factorial design: stress + probiotics group (injection of 2 mg/kg BW dexamethasone and 0.02 g probiotics per bird per day), stress group (injection of 2 mg/kg BW dexamethasone), probiotics group (injection of 2 mg/kg BW saline and 0.02 g probiotics per bird per day), and control group (injection of 2 mg/kg BW saline). Each group consisted of four replicates with 11 broilers per

replicate. The probiotics group and stress + probiotics group received probiotics dissolved in cooled boiled water via drinking water during days 11–14 and 25–28, with controlled water access to ensure consumption within 30 minutes. The stress group and control group received the same volume of cooled boiled water without probiotics under identical water restriction conditions. The stress group and stress + probiotics group were subcutaneously injected with dexamethasone sodium phosphate during days 12–14 and 26–28, while the probiotics group and control group received the same dose of saline.

1.3 Management

The experiment was conducted at the experimental station of Shandong Agricultural University from June 1, 2016, with a duration of 35 days. Broilers were raised in cages and fed a basal diet formulated according to NRC (1994) nutrient requirements for broilers. Diet composition and nutrient levels are presented in Table 1 . The initial temperature was 35°C, which was gradually reduced by 2-3°C weekly until reaching 25°C. Temperature and humidity were adjusted according to growth conditions. Routine immunization and standard management practices were implemented.

1.4.1 Growth Performance

Average body weight, average daily gain, feed-to-gain ratio, mortality rate, and European Performance Index were calculated throughout the experimental period.

1.4.2 Immune Organ Indices

Prior to slaughter, broilers were weighed, and the thymus, spleen, and bursa of Fabricius were collected and weighed to calculate the corresponding organ indices.

1.4.3 Pancreatic Enzyme Activities

The duodenum was longitudinally opened, and chyme was washed away with cold sterile saline. Mucosa was scraped with a glass slide, and enzyme activities of trypsin and lipase were determined using commercial assay kits. Total protein concentration was measured using a total protein (TP) assay kit (all kits purchased from Nanjing Jiancheng Bioengineering Institute), and enzyme activities were calculated accordingly.

1.4.4 Jejunal Morphology

A 2-cm segment of jejunum was collected approximately 5 cm anterior to the yolk stalk and fixed in 4% paraformaldehyde. After fixation, dehydration, clearing, paraffin embedding, and staining, tissue sections were prepared. Jejunal villus

height and crypt depth were measured under a microscope, and the villus height-to-crypt depth ratio was calculated.

1.5 Statistical Analysis

Experimental data were preliminarily processed using Excel 2013 and statistically analyzed using SAS 9.0 software. Two-way ANOVA was performed for significance testing. Results are expressed as means ± standard deviation, with $P<0.05$ considered statistically significant and $P<0.10$ indicating a trend.

2. Results

2.1 Effects of Stress on Growth Performance and the Role of Probiotics

As shown in Table 2 , stress significantly reduced average body weight, average daily gain, and European Performance Index of 35-day-old male broilers ($P<0.05$), while significantly increasing feed-to-gain ratio and mortality rate ($P<0.05$). Probiotics significantly reduced mortality rate ($P<0.05$) and showed a trend toward improving European Performance Index ($P<0.10$).

2.2 Effects of Stress on Immune Organ Indices and the Role of Probiotics

Table 3 shows that stress significantly reduced thymus index, spleen index, and bursa of Fabricius index at both 15 and 29 days of age ($P<0.05$).

2.3 Effects of Stress on Pancreatic Digestive Enzyme Activities and the Role of Probiotics

Table 4 indicates that probiotics significantly increased pancreatic lipase activity at 29 days of age ($P<0.05$), while stress decreased both trypsin and lipase activities at 15 and 29 days of age ($P>0.05$).

2.4 Effects of Stress on Jejunal Morphology and the Role of Probiotics

Table 5 demonstrates that stress significantly reduced jejunal villus height at 15 and 29 days of age and villus height-to-crypt depth ratio at 29 days of age ($P<0.05$), with a trend toward reducing the ratio at 15 days of age ($P<0.10$). Probiotics significantly increased villus height ($P<0.05$) and reduced crypt depth ($P<0.05$) at 29 days of age. A significant interaction between stress and probiotics was observed for villus height-to-crypt depth ratio at both 15 and 29 days of age ($P<0.05$).

3. Discussion

3.1 Effects of Stress on Growth Performance and the Role of Probiotics

Stress represents the sum of non-specific responses to internal and external stimuli. During stress, animals secrete glucocorticoids that act on specific target organs and cells to mobilize energy reserves, increase blood glucose, and enhance resistance. Research indicates that stress promotes hepatic glycogen synthesis and gluconeogenesis while enhancing protein and fat catabolism [7], thereby reducing weight gain and shortening intestinal transit time, which decreases feed utilization efficiency [3]. Our results showed that stress significantly reduced average body weight, average daily gain, and European Performance Index while increasing feed-to-gain ratio, consistent with previous findings and confirming that stress impairs growth performance in male broilers.

Probiotics are known to promote animal growth, improve feed conversion efficiency, and reduce mortality. Xue et al. [8] reported that *Bacillus subtilis* preparations increased average body weight and daily gain in broilers. In this study, probiotics increased average daily gain and European Performance Index by 4.87% and 13.19%, respectively, reduced feed-to-gain ratio by 1.25%, and significantly decreased mortality by 66.67%. These effects likely result from increased beneficial bacteria, improved intestinal environment, and enhanced nutrient digestion and absorption, aligning with previous research. However, the probiotic used in this study only partially alleviated stress-induced reductions in growth performance, possibly due to the specific type and composition of the probiotic preparation.

3.2 Effects of Stress on Immune Organ Indices and the Role of Probiotics

Stress compromises animal immunity and resistance, increasing morbidity and mortality [9]. Selye [10] reported that glucocorticoids secreted during stress cause thymus and lymphoid tissue atrophy, inhibit protein synthesis, and suppress humoral and cellular immunity, weakening disease resistance. Our study demonstrated that stress significantly reduced thymus, spleen, and bursa of Fabricius indices at both 15 and 29 days of age, confirming that stress inhibits immune organ development. Liu et al. [11] similarly showed that stress impairs immune organ development and differentiation, significantly reducing relative weights of thymus, spleen, and bursa in broilers.

Probiotics promote the growth and development of immune cells, tissues, and organs. Ma et al. [12] found that probiotic supplementation significantly increased serum immunoglobulin levels and improved bursa and spleen indices in chicks. Wang [13] reported that *Lactobacillus acidophilus* cell wall extracts enhanced the cytotoxic activity of mouse intestinal intraepithelial lymphocytes. Liu et al. [14] observed significantly increased immune organ weights in chicks fed probiotics, indicating accelerated development and maturation. In our study,

probiotics increased thymus, spleen, and bursa indices by 9.52%, 28.57%, and 7.14% at 15 days, and by 14.29%, 16.67%, and 20.00% at 29 days, respectively, demonstrating that probiotics promote immune organ development. However, the probiotic failed to completely reverse stress-induced inhibition of immune organ development, only partially mitigating the negative effects, which may be attributed to the specific probiotic composition.

3.3 Effects of Stress on Pancreatic Digestive Enzyme Activities and the Role of Probiotics

Changes in digestive enzyme activities result from structural alterations and variations in enzyme secretion induced by external factors [15]. Ruan et al. [16] reported that heat stress significantly decreased intestinal protease and lipase activities in male broilers, with daily gain showing a highly significant positive correlation with these enzyme activities. Wang [17] demonstrated that stress affects digestive enzyme synthesis and secretion by regulating pancreatic enzyme gene expression at the transcriptional level. Our results showed that stress reduced trypsin activity by 8.70% and 18.44% and lipase activity by 12.49% and 1.62% at 15 and 29 days of age, respectively, confirming that stress decreases pancreatic enzyme activities.

Probiotics primarily function by promoting endogenous digestive enzyme secretion and maintaining intestinal microbial balance [18]. Our findings indicated that probiotics increased trypsin activity by 10.46% and 26.70% at 15 and 29 days, respectively, and lipase activity by 11.05% at 29 days, though these differences were not statistically significant. Sögaard and Suhr-Jessen [19] reported that *Bacillus subtilis* and *Bacillus licheniformis* possess strong protease, amylase, and lipase activities. Zhang et al. [20] found that a probiotic mixture containing *Bacillus licheniformis* and lactobacilli significantly increased intestinal protease and lipase activities in chicks. The discrepancy between these significant findings and our results may be related to differences in probiotic composition and type.

3.4 Effects of Stress on Jejunal Morphology and the Role of Probiotics

Intestinal mucosal morphology is a primary factor affecting nutrient absorption [21]. Villus height and villus height-to-crypt depth ratio positively correlate with enhanced nutrient absorption, while increased crypt depth negatively correlates with absorption efficiency [22]. Studies have shown that stress significantly alters intestinal morphology and structure [23], with glucocorticoids affecting mucosal cell development [24]. Hu et al. [25] reported that stress impairs intestinal mucosal growth, reducing jejunal villus height. Hu and Guo [26] found that corticosterone treatment simulating stress decreased intestinal mucosal cell proliferation and hindered normal villus growth. Our results confirmed that stress significantly reduced jejunal villus height in male broilers, consistent with previous research.

Li et al. [27] demonstrated that probiotic supplementation promoted rapid intestinal villus development in broilers. Liu et al. [28] reported that probiotics significantly increased intestinal villus height and reduced crypt depth in early-weaned piglets. In our study, probiotics increased villus height by 3.33% and 33.33% at 15 and 29 days of age, respectively, and significantly reduced crypt depth at 29 days, indicating that probiotics promote intestinal villus development. The significant interaction between probiotics and stress for villus height demonstrates that probiotics can substantially alleviate stress-induced damage to intestinal villi.

Conclusion

1. Stress reduces growth performance, inhibits normal immune organ development, and disrupts intestinal environment and tissue structure in male broilers.
2. Probiotics can significantly alleviate stress-induced damage to intestinal villi but show no significant improvement in growth performance.

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