

## Effects of $\beta$ -Glucan on Growth Performance, Immune Function, and Intestinal Microenvironment in Broiler Chickens (Postprint)

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### Abstract

This experiment aimed to investigate the effects of dietary  $\beta$ -glucan supplementation on growth performance, immune function, and intestinal microenvironment in broiler chickens. A total of 672 one-day-old Arbor Acres (AA) broiler chickens were selected and randomly divided into 4 groups with 14 replicates per group and 12 chickens per replicate. The control group was fed a basal diet, while the experimental groups were fed test diets supplemented with 100, 150, and 200 g/t  $\beta$ -glucan on top of the basal diet. The experimental period lasted 42 days. The results showed: 1) The body weight gain of broiler chickens aged 1-21 days in the 150 g/t dose group was significantly higher than that of the control group ( $P < 0.05$ ). 2) The serum immunoglobulin content of broiler chickens in the 150 g/t dose group was higher than that of the control group ( $P > 0.05$ ). 3) At 21 days of age, the number of Lactobacillus in the cecum of broiler chickens in the 150 g/t dose group was significantly higher than that of the control group ( $P < 0.05$ ), while the number of Salmonella in the jejunum and ileum was significantly lower than that of the control group ( $P < 0.05$ ); at 42 days of age, the number of Salmonella in the cecum of broiler chickens in the 150 and 200 g/t dose groups was significantly lower than that of the control group and the 100 g/t dose group ( $P < 0.05$ ). 4) Dietary  $\beta$ -glucan supplementation had a significant effect on the villus height to crypt depth ratio in the jejunum of 42-day-old broiler chickens ( $P < 0.05$ ). It can be concluded that appropriate dietary supplementation of  $\beta$ -glucan can increase slaughter weight, improve growth performance, increase the number of Lactobacillus in the cecum, and reduce the number of Salmonella in the jejunum, ileum, and cecum of broiler chickens.

## Full Text

### Effects of $\beta$ -Glucan on Growth Performance, Immune Function, and Intestinal Microenvironment of Broiler Chickens

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**Abstract:** This experiment was conducted to investigate the effects of dietary  $\beta$ -glucan supplementation on growth performance, immune function, and intestinal microenvironment in broiler chickens. A total of 672 one-day-old Arbor Acres (AA) broilers were randomly allocated to four groups with 14 replicates per group and 12 birds per replicate. The control group received a basal diet, while the experimental groups received the basal diet supplemented with 100, 150, or 200 g/t  $\beta$ -glucan. The 42-day feeding trial yielded the following results: (1) Body weight gain of broilers aged 1-21 days in the 150 g/t group was significantly higher than that in the control group ( $P < 0.05$ ). (2) Serum immunoglobulin content in the 150 g/t group was elevated compared to the control group ( $P > 0.05$ ). (3) At 21 days of age, the cecal *Lactobacillus* count in the 150 g/t group was significantly higher than in the control group ( $P < 0.05$ ), while *Salmonella* counts in the jejunum and ileum were significantly lower ( $P < 0.05$ ). At 42 days of age, cecal *Salmonella* counts in the 150 and 200 g/t groups were significantly lower than in both the control and 100 g/t groups ( $P < 0.05$ ). (4) Dietary  $\beta$ -glucan supplementation significantly affected the jejunal villus height to crypt depth ratio in 42-day-old broilers ( $P < 0.05$ ). In conclusion, appropriate dietary  $\beta$ -glucan supplementation can improve market weight and growth performance, increase cecal *Lactobacillus* populations, and reduce *Salmonella* populations in the jejunum, ileum, and cecum of broiler chickens.

**Keywords:**  $\beta$ -glucan; broiler chickens; growth performance; immune function; intestinal microenvironment

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## Introduction

$\beta$ -Glucan is a class of non-starch polysaccharides (NSP) widely distributed in bacteria, fungi, yeast, and plants [1]. Research suggests that  $\beta$ -glucan may contain active groups that enhance immune function, acting as a broad-spectrum immunomodulator that promotes immune organ development and strengthens the immune system [2].  $\beta$ -Glucan exhibits various biological activities, including hypolipidemic, hypoglycemic, hepatoprotective, anti-inflammatory, and antiox-

ident effects, and can improve viral resistance in mice [3]. China is rich in  $\beta$ -glucan resources, as the rapid development of the beer and fermentation industries generates substantial yeast sludge containing  $\beta$ -glucan as a fermentation byproduct. Utilizing this resource not only prevents environmental pollution but also creates economic value [4].

$\beta$ -Glucan has demonstrated efficacy in enhancing animal immune function. Sang et al. [5] reported that dietary  $\beta$ -glucan supplementation improved immunity and survival rates in crayfish. Li et al. [6] observed a trend toward improved non-specific immunity in broiler chickens fed  $\beta$ -glucan-supplemented diets. Wu et al. [7] found that  $\beta$ -glucan supplementation enhanced growth performance and ammonia nitrogen stress resistance in Asian seabass. To better understand the potential of  $\beta$ -glucan as a feed additive, this study investigated its effects on growth performance, immune organs, serum immune parameters, and intestinal microbiota in white-feathered broiler chickens.

## Materials and Methods

**1.1 Experimental Design** A total of 672 one-day-old AA broiler chickens with similar body weights were randomly assigned to four groups (one control and three  $\beta$ -glucan-supplemented groups), each comprising 14 replicates of 12 male birds. The 42-day feeding trial was divided into two phases: days 1-21 and days 22-42. All birds received corn-soybean meal-based pelleted diets. The control group was fed the basal diet, while the experimental groups received the basal diet supplemented with 100, 150, or 200 g/t  $\beta$ -glucan (purchased from Algal Scientific Cooperation, MI, USA). Birds were housed in four-tier cages. The composition and nutrient levels of the basal diets are presented in Table 1.

**1.2 Management Practices** The 42-day trial consisted of a starter phase (days 1-21) and a grower phase (days 22-42). Birds were raised in cages with ad libitum access to feed and water. The initial temperature was set at 35°C and gradually reduced by 2-3°C weekly until reaching 23°C. Relative humidity was maintained at 45% and adjusted according to bird growth. A 24-hour lighting program was implemented. Electrolyte multivitamins and glucose were added to drinking water during days 1-3. Routine vaccination protocols were followed, and other management practices conformed to standard commercial broiler production procedures.

**1.3 Sample Collection** On the mornings of days 21 and 42, body weights were recorded for each group before feeding without prior feed withdrawal. Feed consumption and bird weights were measured to calculate body weight gain, feed intake, mortality and culling rate, feed conversion ratio, and European Production Index (EPI) for days 1-21, 22-42, and the overall period (1-42 days). Mortality and culling events were meticulously documented, with deceased or culled birds weighed to adjust calculations periodically.

On days 21 and 42, one bird per replicate was slaughtered to collect the bursa of Fabricius and spleen for weighing. Mixed jejunal and ileal digesta and cecal digesta were collected for microbiological analysis. Jejunal and ileal tissue samples were obtained for histomorphological examination. On days 22 and 42, after weighing and feed measurement, one bird per replicate was selected for blood collection from the wing vein, and serum was separated by centrifugation.

#### 1.4 Analytical Methods 1.4.1 Growth Performance Calculations

Body weight gain (WG) = Total final bird weight + weight of dead/culled birds - initial bird weight

Mortality and culling rate (DR, %) = (Number of dead and culled birds / Total number of birds)  $\times$  100

Feed conversion ratio (F/G) = Total feed consumption / Body weight gain

European Production Index (EPI) = 10,000  $\times$  Average body weight (kg)  $\times$  Survival rate (%) / (Feed conversion ratio  $\times$  Days)

#### 1.4.2 Immune Function Parameters

Relative immune organ weight was expressed as immune organ weight divided by live body weight. Serum immune parameters including endotoxin (ET), immunoglobulin G (IgG), and immunoglobulin A (IgA) were determined using biotin double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kits purchased from Nanjing Jiancheng Bioengineering Institute. Absorbance was measured using an ELx808 microplate reader (Gene Company Limited).

#### 1.4.3 Intestinal Parameters

Lactobacillus and Salmonella counts in mixed jejunal and ileal digesta and cecal digesta were determined using plate count methods. Briefly, 25 g of digesta was placed in a sterile bottle containing 225 mL of sterile physiological saline, vigorously shaken to prepare a 1:10 uniform dilution, and subsequently diluted in 10-fold serial dilutions. Two to three appropriate dilutions were selected, and 1 mL of each dilution was transferred to sterile petri dishes (two plates per dilution). Approximately 15 mL of nutrient agar medium at 46°C was immediately poured into each plate, mixed thoroughly, and allowed to solidify. Plates were inverted and incubated at 37°C for (24 $\pm$ 2) h. Colony counts were multiplied by the dilution factor to obtain the total bacterial count per gram of digesta.

Jejunal and ileal tissue samples were processed into histological sections using a microtome (Leica, Germany). After staining and mounting, villus height and crypt depth were measured at 10 $\times$ 4 magnification using a light microscope (NIKON ECLIPSE-80i) and NIS-Elements D 4.20.00 software. Two non-consecutive sections were examined per sample, with three fields of view per section and ten measurements per field. The mean value served as the final measurement for calculating the villus height to crypt depth ratio (V/C).

**1.5 Statistical Analysis** Data are presented as means  $\pm$  standard deviation. One-way analysis of variance (ANOVA) was performed using SAS 9.1 software. Differences were considered significant at  $P < 0.05$ .

## Results

**2.1 Effects of  $\beta$ -Glucan on Broiler Growth Performance** As shown in Table 2, dietary  $\beta$ -glucan supplementation significantly affected body weight gain and EPI of broilers aged 1–21 days ( $P < 0.05$ ). The 150 g/t group exhibited significantly higher body weight gain compared to the control, 100 g/t, and 200 g/t groups. The EPI increased initially and then decreased with increasing  $\beta$ -glucan levels, reaching its maximum in the 150 g/t group. Additionally,  $\beta$ -glucan supplementation tended to reduce feed conversion ratio ( $P = 0.0879$ ) and mortality rate ( $P = 0.0817$ ).

**Table 2** Growth performance of broilers aged 1–21 days

During days 22–42 (Table 3), dietary  $\beta$ -glucan supplementation had no significant effects on body weight gain, feed intake, feed conversion ratio, or mortality rate ( $P > 0.05$ ).

**Table 3** Growth performance of broilers aged 22–42 days

As presented in Table 4, dietary  $\beta$ -glucan supplementation significantly affected overall mortality rate during days 1–42 ( $P < 0.05$ ). The 150 g/t group showed significantly higher mortality compared to the 100 and 200 g/t groups ( $P < 0.05$ ). No significant effects were observed on body weight gain, feed intake, feed conversion ratio, or EPI ( $P > 0.05$ ).

**Table 4** Growth performance of broilers aged 1–42 days

**2.2 Effects of  $\beta$ -Glucan on Broiler Immune Organ Indices** Table 5 shows that dietary  $\beta$ -glucan supplementation had no significant effects on spleen index or bursa of Fabricius index at either 21 or 42 days of age ( $P > 0.05$ ).

**Table 5** Immune organ indices of broilers at 21 and 42 days of age

**2.3 Effects of  $\beta$ -Glucan on Broiler Serum Immune Parameters** As shown in Table 6, dietary  $\beta$ -glucan supplementation had no significant effects on serum endotoxin, IgG, or IgA concentrations at either 21 or 42 days of age ( $P > 0.05$ ).

**Table 6** Serum immune parameters of broilers at 21 and 42 days of age

**2.4 Effects of  $\beta$ -Glucan on Broiler Intestinal Microbiota** At 21 days of age (Table 7), the 200 g/t group exhibited significantly higher *Lactobacillus* counts in the jejunum and ileum compared to the 100 and 150 g/t groups ( $P < 0.05$ ). The 150 g/t group showed significantly higher cecal *Lactobacillus* counts compared to the control and 100 g/t groups ( $P < 0.05$ ). At 42 days of

age, dietary  $\beta$ -glucan supplementation significantly affected *Lactobacillus* counts in the jejunum and ileum ( $P < 0.05$ ) but not in the cecum ( $P > 0.05$ ). The 200 g/t group had significantly higher jejunal and ileal *Lactobacillus* counts than the control group ( $P < 0.05$ ).

**Table 7** Intestinal *Lactobacillus* counts of broilers at 21 and 42 days of age

As shown in Table 8, at 21 days of age, dietary  $\beta$ -glucan supplementation significantly affected *Salmonella* counts in the jejunum and ileum ( $P < 0.05$ ) but not in the cecum ( $P > 0.05$ ). The 150 g/t group exhibited significantly lower jejunal and ileal *Salmonella* counts compared to the control group ( $P < 0.05$ ). At 42 days of age, dietary  $\beta$ -glucan supplementation had no significant effect on jejunal and ileal *Salmonella* counts ( $P > 0.05$ ) but significantly affected cecal *Salmonella* counts ( $P < 0.05$ ). The 150 and 200 g/t groups showed significantly lower cecal *Salmonella* counts than both the control and 100 g/t groups ( $P < 0.05$ ).

**Table 8** Intestinal *Salmonella* counts of broilers at 21 and 42 days of age

**2.5 Effects of  $\beta$ -Glucan on Small Intestinal V/C Ratio** As presented in Table 9, dietary  $\beta$ -glucan supplementation had no significant effects on jejunal or ileal V/C ratios at 21 days of age ( $P > 0.05$ ). At 42 days of age, the 200 g/t group exhibited a significantly higher jejunal V/C ratio than the 150 g/t group ( $P < 0.05$ ), though overall effects remained non-significant ( $P > 0.05$ ).

**Table 9** Villus height to crypt depth ratio (V/C) of small intestine in broilers at 21 and 42 days of age

## Discussion

**3.1 Effects of  $\beta$ -Glucan on Broiler Growth Performance** The present results demonstrate that dietary  $\beta$ -glucan supplementation at 150 g/t improved market weight and growth performance in broiler chickens. Previous reports indicate that  $\beta$ -glucan, as a non-starch polysaccharide, can increase intestinal viscosity, impair nutrient absorption, and consequently inhibit animal growth [8]. However, because  $\beta$ -glucan is poorly absorbed in the gastrointestinal tract, resident probiotic bacteria can utilize it to promote their own growth and maintain intestinal health [9]. These opposing mechanisms likely explain why the 100 and 200 g/t groups showed no significant differences from the control group. The superior body weight gain and market weight observed in the 150 g/t group suggest that this level of supplementation is beneficial for broiler performance, while higher inclusion rates (200 g/t) may exert adverse effects on production.

**3.2 Effects of  $\beta$ -Glucan on Broiler Immune Function** This study found that the 150 g/t group exhibited a higher bursa of Fabricius index at 21 days of age compared to the control, though no significant differences were observed at 42 days. These findings align with those of Liu et al. [10] in broiler chickens, suggesting that  $\beta$ -glucan may modulate immune function by influencing bursal development. The bursa of Fabricius plays a critical role in humoral immunity

and B lymphocyte function during early development, indicating that  $\beta$ -glucan supplementation at 150 g/t may effectively enhance immune competence in young broilers.

Additionally, the 150 g/t group showed increased serum IgG concentrations, consistent with findings in calves [11] and goats [12]. IgG is a major immunoglobulin involved in humoral immune responses, with functions including antigen binding, complement activation, and immune regulation. These results suggest that  $\beta$ -glucan supplementation may enhance humoral immunity in broiler chickens, though the specific implications of elevated serum IgG in the absence of pathogen challenge require further investigation.

**3.3 Effects of  $\beta$ -Glucan on Broiler Intestinal Microenvironment** This study demonstrated that moderate  $\beta$ -glucan supplementation (150 or 200 g/t) promoted *Lactobacillus* growth while inhibiting *Salmonella* proliferation in the intestine. Pan et al. [13] similarly observed a trend toward increased cecal *Lactobacillus* populations with  $\beta$ -glucan supplementation. As the first line of defense, a healthy gastrointestinal microbiota is essential for optimal animal performance. The observed effects on intestinal morphology and microbiota composition warrant further investigation to elucidate their full implications.

The 200 g/t group exhibited the highest jejunal V/C ratio at 42 days of age, while the 150 g/t group showed a significantly lower V/C ratio than the control group. This may be attributed to the anti-nutritional effects of  $\beta$ -glucan becoming more pronounced at higher inclusion levels, particularly at 150 g/t. The small intestinal mucosal structure forms the anatomical basis for nutrient digestion and absorption, with the V/C ratio serving as a comprehensive indicator of intestinal function [14]. Villus atrophy, characterized by reduced villus height and increased crypt depth, impairs nutrient absorption. Conversely, an increased V/C ratio expands the absorptive surface area and enhances digestive capacity. Dietary  $\beta$ -glucan supplementation at 200 g/t appeared to strengthen jejunal digestive function in broiler chickens.

## Conclusion

Appropriate dietary  $\beta$ -glucan supplementation can improve market weight and growth performance, increase cecal *Lactobacillus* populations, and reduce *Salmonella* counts in the jejunum, ileum, and cecum of broiler chickens.

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