

Effects of Exopolysaccharide from *Gynostemma pentaphyllum* Endophytic Fungus JY25 on Growth Performance and Intestinal Mucosal Immune Function in Layer Chicks Postprint

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Abstract

The experiment aimed to investigate the effects of exopolysaccharide from endophytic fungus JY25 of *Gynostemma pentaphyllum* (JY25P) on growth performance and intestinal mucosal immune function in layer chicks. Three hundred healthy 1-day-old Sanhuang layer chicks were randomly divided into 5 groups (with 3 replicates per group and 20 chicks per replicate): Group I was the blank control group administered sterile water; Groups II, III, and IV were experimental groups administered 0.04%, 0.08%, and 0.12% JY25P, respectively; Group V was the positive control group administered 0.08% Astragalus polysaccharide. The results showed: 1) Compared with Group I, Group IV exhibited a significant increase in average daily gain by 15.86% ($P < 0.05$) and a highly significant reduction in feed-to-gain ratio by 24.13% ($P < 0.01$). 2) At all experimental stages, Groups III, IV, and V significantly elevated Newcastle disease antibody titers in serum of layer chicks ($P < 0.05$). 3) At 15 and 20 days of age, Group IV highly significantly increased intestinal mucosal interleukin-4 (IL-4) and interferon- γ (IFN- γ) levels ($P < 0.01$), while exerting no significant effect on secretory immunoglobulin A (SIgA) or IFN- γ /IL-4 ratio ($P > 0.05$). These results indicate that oral administration of 0.12% JY25P can improve growth performance, enhance specific Newcastle disease antibody titers, promote non-specific secretion of intestinal mucosal IL-4 and IFN- γ , and maintain immune homeostasis in layer chicks; therefore, this polysaccharide demonstrates promising application prospects.

Full Text

Effects of Extracellular Polysaccharide from *Gynostemma pentaphyllum* Endophytic Fungi JY25 on Growth Performance and Intestinal Mucosal Immune Function in Laying Chicks

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Abstract

This experiment investigated the effects of extracellular polysaccharide from *Gynostemma pentaphyllum* endophytic fungi JY25 (JY25P) on growth performance and intestinal mucosal immune function in laying chicks. Three hundred healthy one-day-old Sanhuang laying chicks were randomly divided into five groups with three replicates per group and 20 chicks per replicate. Group 1 served as the blank control receiving sterile water; groups 2, 3, and 4 were experimental groups receiving 0.04%, 0.08%, and 0.12% JY25P, respectively; and group 5 was the positive control receiving 0.08% Astragalus polysaccharide. The results showed: 1) Compared with group 1, the average daily gain of group 4 increased significantly by 15.86% ($P < 0.05$), while the feed/gain ratio decreased extremely significantly by 24.13% ($P < 0.01$). 2) Throughout all experimental stages, groups 2, 3, and 4 significantly increased Newcastle disease antibody titers in serum ($P < 0.05$). 3) At 15 and 20 days of age, group 4 extremely significantly increased intestinal mucosal interleukin-4 (IL-4) and interferon- γ (IFN- γ) contents ($P < 0.01$), while showing no significant effect on secretory immunoglobulin A (SIgA) or the IFN- γ /IL-4 ratio ($P > 0.05$). These findings indicate that oral administration of 0.12% JY25P improves growth performance, enhances specific Newcastle disease antibody titers, boosts non-specific intestinal mucosal IL-4 and IFN- γ secretion, and maintains immune homeostasis, demonstrating promising application potential for this polysaccharide.

Keywords: JY25 exopolysaccharides; laying chicks; growth performance; intestinal mucosal immunity; Newcastle disease antibody titer

Fungal polysaccharides are natural high-molecular-weight polymers composed of more than ten monosaccharides linked by glycosidic bonds, extracted from fungal fruiting bodies, mycelium, and fermentation broth. These compounds have attracted widespread attention due to their potent immunomodulatory activities. Compared with plant polysaccharides, extracellular fungal polysaccharides offer several advantages: they are not limited by seasonal, geographical, pest, or growth cycle constraints; they are easily separated and can be produced

through continuous fermentation; and they exhibit high efficiency, low toxicity, immune-enhancing, antimicrobial, antiviral, and residue-free properties. Consequently, they have become a focal point for researchers worldwide. As novel green feed additives, fungal polysaccharides can be formulated into pellet feeds or mixed with feed as powder. Studies have demonstrated that incorporating fungal polysaccharides into feed can regulate and maintain intestinal flora balance, enhance immunity, and promote livestock growth, showing broad application prospects in animal husbandry. Liu et al. reported that dietary supplementation with lentinan improved growth performance and feed conversion ratio in broilers while increasing immune organ indices. Wang et al. found that oral administration of 40 mg/kg *Ganoderma* polysaccharide accelerated antibody production and significantly enhanced antibody levels in Arbor Acre broilers, markedly improving immune capacity. However, the application of extracellular polysaccharides from medicinal plant endophytic fungi in livestock feed remains unreported. Therefore, this study evaluated JY25P in laying chicks to investigate its effects on growth performance and intestinal mucosal immune function, providing scientific evidence for its use as a green feed additive. Given the short experimental period, small chick size, and unstable feed intake, oral gavage was employed to ensure consistent polysaccharide intake.

1.1 Experimental Design

Three hundred healthy one-day-old Sanhuang laying chicks were raised to six days of age before initiating the experiment. The chicks were randomly divided into five groups with three replicates each containing 20 birds. Group served as the blank control; groups , , and were experimental groups receiving JY25P at 0.04%, 0.08%, and 0.12% of body weight, respectively; and group was the positive control receiving 0.08% *Astragalus* polysaccharide. Administration occurred daily at 08:00.

1.2 Experimental Materials and Diet Composition

Sanhuang chicks were purchased from Luohe Sunshine Poultry Industry Co., Ltd. *Astragalus* polysaccharide (70% purity) was obtained from Shengtaier Biotechnology Group. JY25P was extracted from fermentation broth of the patented strain JY25 (patent number: JN180937.1) preserved in the Physiology Laboratory of Henan University of Technology's College of Biological Engineering, with a polysaccharide content of 69.44%. ELISA kits for secretory immunoglobulin A (SIgA), interleukin-4 (IL-4), and interferon- γ (IFN- γ) were purchased from Boxing Biotechnology Co., Ltd.

The basal diet was PQ-2018 layer starter feed (1-21 days) from Henan Guang'an (Group) Biotechnology Co., Ltd. The composition and nutrient levels are presented in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis), %

Footnotes:

- 1) Provided per kilogram of diet: Cu 10 mg, I 0.75 mg, Fe 100 mg, Mn 60 mg, Se 0.3 mg, Zn 90 mg.
- 2) Provided per kilogram of diet: VA 10,000 IU, VD₃ 3,500 IU, VE 30 mg, VK 1.5 mg, VB₁ 2.9 mg, VB₂ 6.4 mg, VB₆ 2.5 mg, VB₁₂ 0.05 mg, biotin 0.08 mg, folic acid 1.0 mg, pantothenic acid 12.0 mg, nicotinic acid 35 mg, ethoxyquin 200 mg.
- 3) Metabolizable energy (ME) was calculated; other values were measured.

1.3.1 Growth Performance

Daily feed intake was recorded throughout the experiment. On days 5, 10, and 15, chicks were fasted for 12 hours and weighed to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed/gain ratio (F/G).

1.3.2 Immune Function

On days 5, 10, and 15, three chicks per replicate were randomly selected to determine specific Newcastle disease antibody titers and intestinal mucosal SIgA, IL-4, and IFN- γ contents.

Newcastle disease antibody titer: Blood was collected from wing veins and serum was separated. Antibody titers were measured using the micro-hemagglutination inhibition assay according to the national standard GB/T 16550–2008 for Newcastle disease diagnosis, representing specific immune status.

Intestinal mucosal SIgA, IL-4, and IFN- γ determination: A 3-cm segment posterior to the duodenum and anterior to the jejunum was excised, weighed, and scraped to obtain mucosa. The sample was rinsed with 2 mL phosphate-buffered saline (pH 7.4) containing 0.01 mmol/L phenylmethylsulfonyl fluoride (PMSF), centrifuged at 5,000 r/min for 5 minutes, and the supernatant was collected. SIgA, IL-4, and IFN- γ contents per unit mass of intestinal mucosa were determined using ELISA kits according to manufacturer instructions, and the IFN- γ /IL-4 ratio was calculated to assess non-specific immune status.

1.4 Statistical Analysis

Data were analyzed using SPSS 19.0 software via one-way ANOVA, with Duncan's multiple comparison test applied when significant differences were detected. Significance was set at $P < 0.05$ and extreme significance at $P < 0.01$. Results are expressed as "mean \pm standard error."

2.1 Effects of JY25P on Growth Performance of Laying Chicks

As shown in Table 2, JY25P administration affected ADG, ADFI, and F/G to varying degrees. Compared with group , ADG in group increased significantly by 7.61% during days 11–15 ($P < 0.05$). During days 16–20, ADG in groups ,

, and increased by 20.43%, 15.46%, and 29.01%, respectively ($P < 0.05$), with no significant differences among these three groups ($P > 0.05$). Groups , , and showed significantly higher ADFI than group during days 16-20 ($P < 0.05$). Groups and exhibited extremely significant F/G reductions during days 6-10 and 11-15 ($P < 0.01$), while group maintained this effect during days 16-20 ($P < 0.01$). Overall, group demonstrated the optimal growth-promoting effects. Compared with group , group showed a 10.89% increase in final body weight ($P < 0.05$), 15.86% improvement in ADG ($P < 0.05$), and 24.13% reduction in F/G ($P < 0.01$) during days 6-20, indicating JY25P outperformed Astragalus polysaccharide.

Table 2 Effects of JY25P on growth performance of laying chicks

Note: In the same column, values with the same or no letter superscripts indicate no significant difference ($P > 0.05$), different lowercase letters indicate significant difference ($P < 0.05$), and different uppercase letters indicate extreme significant difference ($P < 0.01$). The same applies below.

2.2 Effects of JY25P on Newcastle Disease Antibody Titer

Table 3 shows that groups , , and significantly increased serum Newcastle disease antibody titers compared with group at all stages ($P < 0.05$), with improvements of 9.47%, 14.32%, and 10.57%, respectively, at 20 days of age. These results demonstrate that JY25P effects on Newcastle disease antibody titers are comparable to those of Astragalus polysaccharide.

Table 3 Effects of JY25P on Newcastle disease antibody titer of laying chicks (\log_2)

2.3 Effects of JY25P on Intestinal Mucosal Immune Function

Table 4 reveals that JY25P and Astragalus polysaccharide had no significant effect on intestinal mucosal SIgA secretion ($P > 0.05$) at any stage. Compared with group , IL-4 content was not significantly affected at 10 days of age ($P > 0.05$), but increased extremely significantly in groups and at 15 days of age by 2.36% and 2.46%, respectively ($P < 0.01$), and in groups and at 20 days of age by 2.97% and 2.69%, respectively ($P < 0.01$). These findings indicate that 0.08% and 0.12% JY25P doses progressively increased intestinal mucosal IL-4 content with prolonged administration.

IFN- γ content showed no significant changes at 10 days of age ($P > 0.05$), but the promoting effect became increasingly evident over time. At 15 days of age, group exhibited a 2.38% increase ($P < 0.01$); at 20 days of age, groups and increased by 2.56% and 2.86%, respectively ($P < 0.01$). Thus, the 0.12% JY25P dose proved most effective for enhancing intestinal mucosal IFN- γ content.

Notably, group significantly reduced the IFN- γ /IL-4 ratio at 15 and 20 days of age ($P < 0.05$), whereas JY25P had no significant effect on this ratio throughout the experiment ($P > 0.05$).

Table 4 Effects of JY25P on SIgA, IL-4, and IFN- γ contents in intestinal mucosa of laying chicks

3.1 Effects of JY25P on Growth Performance of Laying Chicks

Previous studies have shown that dietary polysaccharide supplementation effectively enhances appetite and promotes animal growth, primarily by stimulating intestinal development, increasing beneficial gut microbiota, improving nutrient absorption efficiency, accelerating protein synthesis, and thereby optimizing growth performance. Ge et al. reported that dietary supplementation with 0.3% *Agaricus blazei* polysaccharide increased ADG by 6.49%, ADFI by 3.68%, and reduced F/G by 3.11% in AA broilers, consistent with our findings. Our results demonstrate that 0.12% JY25P administration yielded superior growth performance compared with Astragalus polysaccharide, possibly because Astragalus polysaccharide is a plant-derived polysaccharide with different growth-promoting mechanisms and structural functional groups than fungal polysaccharides, leading to differential effects on growth performance.

3.2 Effects of JY25P on Newcastle Disease Antibody Titer in Laying Chicks

Newcastle disease antibody titer reflects both resistance to Newcastle disease virus and specific humoral immune capacity. Liu et al. reported that dietary supplementation with 0.8% *Ganoderma* polysaccharide increased Newcastle disease antibody titers by 4.80% in Avian chicks. In our study, groups , , and increased antibody titers by 9.47%, 14.32%, and 10.57%, respectively, at 20 days of age compared with the blank control, aligning with the immunostimulatory effects of *Ganoderma* polysaccharide. Moreover, JY25P demonstrated comparable efficacy to Astragalus polysaccharide, whose active components are known to stimulate the immune system, maintain high immune activity, prolong B lymphocyte antibody secretion, delay antibody titer decline, and enhance Newcastle disease antibody levels. Therefore, we conclude that JY25P can improve specific immune performance and disease resistance in laying chicks.

3.3 Effects of JY25P on Intestinal Mucosal Immune Function in Laying Chicks

Intestinal mucosal immunity is primarily mediated by SIgA, which prevents pathogen invasion and functions as specific mucosal immunity. IL-4, a characteristic cytokine produced by T helper 2 (Th2) cells, acts on B lymphocytes, inhibits Th1 cytokine production, serves as an effective anti-inflammatory factor, and enhances humoral immunity. IFN- γ , mainly produced by Th1 and natural killer (NK) cells, induces interleukin-2 (IL-2) receptor expression, thereby stimulating T lymphocyte proliferation and enhancing cellular immune responses. The IFN- γ /IL-4 ratio reflects Th cell differentiation and indicates whether the regulation of cellular and humoral immunity by Th1 and Th2 cells is balanced.

Our results showed that JY25P had no significant effect on intestinal mucosal IL-4 and IFN- γ contents during the early experimental stage. However, with prolonged administration, 0.12% JY25P activated and promoted Th2 cell secretion of IL-4, thereby enhancing intestinal mucosal humoral immunity. Simultaneously, it increased IFN- γ content, strengthened antigen-presenting cell-T lymphocyte interactions, induced IL-2 receptor expression, promoted T lymphocyte proliferation, and enhanced cellular immune responses. Furthermore, JY25P did not significantly affect the IFN- γ /IL-4 ratio, indicating that it stimulates both Th1 and Th2 cell subsets, enhances both humoral and cellular immunity, and maintains dynamic immune balance.

In summary: JY25P significantly improves ADG and reduces F/G in laying chicks, enhancing growth performance and economic value. JY25P increases Newcastle disease antibody titers to enhance specific immunity and disease resistance, while promoting IL-4 and IFN- γ secretion in intestinal mucosa to improve both humoral and cellular immunity, maintain mucosal immune balance, and strengthen non-specific immunity.

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