

## Effects of *Pseudostellaria heterophylla* Stem and Leaf Polysaccharide on Growth Performance and Serum Antioxidant, Immune, and Biochemical Indices in Weaned Piglets: Postprint

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### Abstract

This experiment aimed to investigate the effects of dietary supplementation of *Pseudostellaria heterophylla* stem and leaf polysaccharide on growth performance, serum antioxidant indices, immune indices, and biochemical indices of weaned piglets. One hundred and twenty 25-day-old “Duroc × Landrace” weaned piglets were randomly allocated to 4 groups (control group, group I, group II, group III), with 3 replicates per group and 10 piglets per replicate. The basal diet was supplemented with 0, 500, 1,000, and 1,500 mg/kg of *Pseudostellaria heterophylla* stem and leaf polysaccharide, respectively, for a trial period of 30 days. The results showed that, compared with the control group: 1) The average daily gain (ADG) of weaned piglets in groups I and II was extremely significantly increased ( $P < 0.01$ ), and the diarrhea rate in groups I, II, and III was extremely significantly decreased ( $P < 0.01$ ). 2) Serum superoxide dismutase (SOD) activity in groups II and III was extremely significantly or significantly increased ( $P < 0.01$  or  $P < 0.05$ ). 3) Serum immunoglobulin A (IgA), complement 3 (C3), and complement 4 (C4) contents in groups I, II, and III were extremely significantly increased ( $P < 0.01$ ), and serum immunoglobulin M content was significantly increased ( $P < 0.05$ ); serum immunoglobulin G (IgG) content in groups II and III was significantly increased ( $P < 0.05$ ). 4) Serum triglyceride (TG) content in groups I, II, and III was extremely significantly decreased ( $P < 0.01$ ); serum urea nitrogen (UN) content in groups II and III was extremely significantly decreased ( $P < 0.01$ ), and serum albumin (ALB) content was extremely significantly increased ( $P < 0.01$ ). The results indicate that dietary supplementation of *Pseudostellaria heterophylla* stem and leaf polysaccharide can effectively improve ADG, reduce diarrhea rate, enhance immune function, and improve antioxidant capacity and serum biochemical indices in weaned piglets. Under the

conditions of this experiment, the appropriate supplementation level of *Pseudostellaria heterophylla* stem and leaf polysaccharide in weaned piglet diets is 1,000 mg/kg.

## Full Text

### Effects of *Radix pseudostellariae* Stem and Leaf Polysaccharide on Growth Performance and Serum Antioxidant, Immune, and Biochemical Indexes in Weaned Piglets

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#### Abstract

This study investigated the effects of dietary *Radix pseudostellariae* stem and leaf polysaccharide (RPSLP) on growth performance and serum antioxidant, immune, and biochemical indexes in weaned piglets. One hundred twenty 25-day-old “Large White × Landrace” weaned piglets were selected and randomly allocated into four groups (control, Group I, Group II, and Group III) with three replicates per group and ten piglets per replicate. The basal diet was supplemented with 0, 500, 1,000, and 1,500 mg/kg RPSLP for a 30-day experimental period. The results showed that, compared with the control group: (1) average daily gain (ADG) in Groups I and II was significantly increased ( $P < 0.01$ ), while diarrhea rate in Groups I, II, and III was significantly decreased ( $P < 0.01$ ); (2) serum superoxide dismutase (SOD) activity in Groups II and III was significantly increased ( $P < 0.01$  or  $P < 0.05$ ); (3) serum immunoglobulin A (IgA), complement 3 (C3), and complement 4 (C4) contents in Groups I, II, and III were significantly increased ( $P < 0.01$ ), and serum immunoglobulin M (IgM) content was significantly increased ( $P < 0.05$ ), while serum immunoglobulin G (IgG) content in Groups II and III was significantly increased ( $P < 0.05$ ); and (4) serum triglyceride (TG) content in Groups I, II, and III was significantly decreased ( $P < 0.01$ ), serum urea nitrogen (UN) content in Groups II and III was significantly decreased ( $P < 0.01$ ), and serum albumin (ALB) content was significantly increased ( $P < 0.01$ ). These findings indicate that dietary supplementation with RPSLP can effectively improve ADG, reduce diarrhea rate, enhance immune function, and improve antioxidant capacity and serum biochemical profiles in weaned piglets. Under the conditions of this experiment, the appropriate supplemental level of RPSLP in weaned piglet diets is 1,000 mg/kg.

**Keywords:** *Radix pseudostellariae* stem and leaf polysaccharide; weaned piglets; growth performance; antioxidant indexes; immune indexes; biochemical indexes

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## Introduction

Weaned piglets often exhibit poor appetite, slow growth, low feed utilization, and diarrhea due to weaning stress. Dietary supplementation with traditional Chinese medicine polysaccharides has been shown to effectively improve growth performance and immune function while reducing diarrhea rates in piglets. Radix pseudostellariae, the dried root tuber of *Pseudostellaria heterophylla* (Miq.) Pax et Hoffm (Caryophyllaceae), possesses anti-stress and immune-enhancing properties attributed to its polysaccharide content. Primarily cultivated in East, Central, North, and Northwest China, with significant production in Fujian, Shandong, Jiangsu, and Anhui provinces, Zherong County in Fujian alone accounts for over 60% of national production, yielding more than 4,900 tons annually. However, most Radix pseudostellariae stems and leaves are discarded or burned, representing substantial waste and environmental pollution. Previous reports indicate that feeding Radix pseudostellariae stems and leaves to piglets can enhance anti-stress capacity and antioxidant function, with polysaccharide content in these aerial parts reaching 5-10%. Nevertheless, the application of purified Radix pseudostellariae stem and leaf polysaccharide in weaned piglets remains largely unexplored. This study examined the effects of RPSLP supplementation on growth performance and serum antioxidant, immune, and biochemical indexes to elucidate its potential health benefits and mechanisms of action, thereby providing a scientific basis for its application in weaned piglets and offering data to support further development of Radix pseudostellariae stems and leaves.

## Materials and Methods

### 1.1 Experimental Material

Radix pseudostellariae stem and leaf polysaccharide, a powdered product purchased from Shaanxi Tengmai Biotechnology Co., Ltd., contained 70% polysaccharides as determined by the phenol-sulfuric acid method using glucose as the standard.

### 1.2 Main Instruments

The experimental instruments included: RT-9900 semi-automatic biochemical analyzer (Shenzhen Rayto Life Sciences Co., Ltd.), VIS-7200 visible spectrophotometer (Shanghai Mapada Instrument Co., Ltd.), OLYMPUS AU2700 automatic chemical and immune analyzer (OLYMPUS Corporation), Sk-1 rapid mixer (Jintan Kexi Instrument Co., Ltd.), and HH-2 digital display constant temperature water bath (Guohua Electrical Appliance Co., Ltd.).

### 1.3 Experimental Animals and Design

One hundred twenty 25-day-old weaned piglets with similar parity and body weight were selected and randomly divided into four groups: control group (basal diet only), Group I (basal diet + 500 mg/kg RPSLP), Group II (basal diet + 1,000 mg/kg RPSLP), and Group III (basal diet + 1,500 mg/kg RPSLP). Each group comprised three replicates with ten piglets per replicate. All piglets had ad libitum access to dry mash feed and water throughout the experiment. During the 5-day pre-trial period, piglets were fed the basal diet and managed according to routine farm procedures for deworming and vaccination. The formal experimental period lasted 30 days. The composition and nutrient levels of the basal diet are presented in Table 1 .

### 1.4 Sample Collection

At the end of the experiment, three piglets with body weight close to the group mean were randomly selected from each replicate. Blood samples (4-5 mL) were collected via anterior vena cava puncture, centrifuged at 3,000 rpm for 10 minutes, and the serum was harvested, aliquoted into Eppendorf tubes, and stored at -20 °C for subsequent analysis.

### 1.5 Measurement Indicators

**1.5.1 Growth Performance** Piglets were individually weighed after fasting at the beginning and end of the experiment to calculate average daily gain (ADG). Daily feed intake was recorded to determine average daily feed intake (ADFI) and feed-to-gain ratio (F/G) for each group. Piglets were observed daily for mental status and fecal consistency, with the number of diarrheic piglets recorded to calculate diarrhea rate using the formula: Diarrhea rate (%) =  $100 \times [(\text{number of diarrheic piglets} \times \text{diarrhea days}) / (\text{total piglets} \times \text{experimental days})]$ .

**1.5.2 Serum Antioxidant Indexes** Serum superoxide dismutase (SOD) activity was measured by xanthine oxidase method, malondialdehyde (MDA) content by thiobarbituric acid method, total antioxidant capacity (T-AOC) by  $\text{Fe}^{3+}$  reduction method, and catalase (CAT) and glutathione peroxidase (GSH-Px) activities by colorimetric assay. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute, and measurements were performed using a VIS-7200 visible spectrophotometer according to the manufacturer' s instructions.

**1.5.3 Serum Immune Indexes** Serum immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin A (IgA), complement 3 (C3), and complement 4 (C4) contents were determined by immunoturbidimetry using assay kits from Zhejiang Yilikang Biotechnology Co., Ltd. and an RT-9900 semi-automatic biochemical analyzer following the manufacturer' s protocols.

**1.5.4 Serum Biochemical Indexes** Serum triglyceride (TG), total protein (TP), albumin (ALB), creatinine (CREA), and urea nitrogen (UN) contents, as well as alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities were analyzed with assistance from the Laboratory Department of Fuzhou Air Force Hospital using assay kits from Beijing Leadman Biochemistry Co., Ltd. and an OLYMPUS AU2700 automatic chemical and immune analyzer.

## 1.6 Statistical Analysis

Experimental data were expressed as “mean  $\pm$  standard deviation” and analyzed by one-way ANOVA using SPSS 17.0 software. Multiple comparisons were performed using the LSD method. Differences were considered significant at  $P < 0.05$  and highly significant at  $P < 0.01$ .

## Results

### 2.1 Effects of *Radix pseudostellariae* Stem and Leaf Polysaccharide on Growth Performance of Weaned Piglets

As shown in Table 2, compared with the control group, ADG in Groups I, II, and III increased by 14.40% ( $P < 0.01$ ), 18.54% ( $P < 0.01$ ), and 6.18% ( $P > 0.05$ ), respectively, with Group II showing significantly higher ADG than Group III ( $P < 0.05$ ). Diarrhea rate in Groups I, II, and III decreased by 58.89% ( $P < 0.01$ ), 64.72% ( $P < 0.01$ ), and 55.97% ( $P < 0.01$ ), respectively, though no significant differences were observed among the three treatment groups ( $P > 0.05$ ). No significant differences were detected in ADFI or F/G among Groups I, II, and III ( $P > 0.05$ ).

### 2.2 Effects of *Radix pseudostellariae* Stem and Leaf Polysaccharide on Serum Antioxidant Indexes of Weaned Piglets

Table 3 shows that serum SOD activity in Groups I, II, and III increased by 9.41% ( $P > 0.05$ ), 18.68% ( $P < 0.01$ ), and 14.12% ( $P < 0.05$ ), respectively, compared with the control group, though no significant differences were observed among the treatment groups ( $P > 0.05$ ). Serum MDA content and CAT and GSH-Px activities, as well as T-AOC, showed no significant differences among all groups ( $P > 0.05$ ).

### 2.3 Effects of *Radix pseudostellariae* Stem and Leaf Polysaccharide on Serum Immune Indexes of Weaned Piglets

As presented in Table 4, serum IgA content in Groups I, II, and III increased by 41.92% ( $P < 0.01$ ), 60.40% ( $P < 0.01$ ), and 58.04% ( $P < 0.01$ ), respectively; serum IgG content increased by 8.17% ( $P > 0.05$ ), 9.57% ( $P < 0.05$ ), and 10.55% ( $P < 0.05$ ), respectively; serum IgM content increased by 17.53% ( $P < 0.05$ ), 19.36% ( $P < 0.05$ ), and 21.39% ( $P < 0.05$ ), respectively; serum C3 content

increased by 26.61% ( $P < 0.01$ ), 38.89% ( $P < 0.01$ ), and 33.78% ( $P < 0.01$ ), respectively; and serum C4 content increased by 37.72% ( $P < 0.01$ ), 53.96% ( $P < 0.01$ ), and 56.91% ( $P < 0.01$ ), respectively. No significant differences were observed in any immune indexes among the three treatment groups ( $P > 0.05$ ).

#### **2.4 Effects of Radix pseudostellariae Stem and Leaf Polysaccharide on Serum Biochemical Indexes of Weaned Piglets**

Table 5 demonstrates that serum TG content in Groups I, II, and III decreased by 22.06% ( $P < 0.01$ ), 33.82% ( $P < 0.01$ ), and 30.88% ( $P < 0.01$ ), respectively, compared with the control group. Serum UN content decreased by 18.21% ( $P < 0.05$ ), 19.20% ( $P < 0.01$ ), and 22.84% ( $P < 0.01$ ), respectively, while serum ALB content increased by 2.10% ( $P > 0.05$ ), 13.14% ( $P < 0.01$ ), and 11.07% ( $P < 0.01$ ), respectively. Group I showed significantly lower serum ALB content compared with Groups II and III ( $P < 0.05$ ). No significant differences were observed in serum TP or CREA contents or ALP, AST, and ALT activities among Groups I, II, and III ( $P > 0.05$ ).

### **Discussion**

#### **3.1 Effects of Radix pseudostellariae Stem and Leaf Polysaccharide on Growth Performance of Weaned Piglets**

Dietary supplementation with traditional Chinese medicine polysaccharides can effectively stimulate the gastrointestinal tract, promote digestive enzyme secretion and nutrient absorption, increase ADG, and support healthy growth in piglets. Previous studies have demonstrated that lentinan polysaccharide can increase feed intake and ADG, while *Acanthopanax senticosus* polysaccharide can enhance nutrient digestibility and significantly improve growth performance. Additionally, polysaccharide supplementation can effectively reduce diarrhea rates. The present results indicate that RPSLP supplementation increased ADFI and ADG while significantly reducing diarrhea rate, suggesting similar functional properties to other polysaccharides in promoting nutrient digestion and growth performance. However, the dose-response relationship was not linear, possibly because RPSLP has a slightly bitter taste that may reduce feed palatability at higher concentrations, thereby decreasing feed intake. Furthermore, excessive polysaccharide doses may reduce nutrient digestibility by binding water and increasing chyme viscosity, which impedes enzyme-substrate interactions and slows nutrient solubilization, ultimately decreasing feed utilization efficiency.

#### **3.2 Effects of Radix pseudostellariae Stem and Leaf Polysaccharide on Serum Antioxidant Indexes of Weaned Piglets**

Metabolic processes generate free radicals that can oxidize unsaturated lipids in biological membranes, forming lipid peroxides whose end product is MDA.

Therefore, MDA content reflects the degree of oxidative damage. The antioxidant defense system comprises enzymatic and non-enzymatic mechanisms, with key enzymes including SOD, GSH-Px, and CAT. SOD plays a crucial role in scavenging free radicals and preventing biomolecular damage. Traditional Chinese medicine polysaccharides exert antioxidant effects by activating specific cellular antibodies, upregulating antioxidant transcription factors, and enhancing antioxidant enzyme expression to neutralize excess free radicals. The current findings show that RPSLP supplementation increased serum SOD activity and decreased MDA content, suggesting that RPSLP may enhance antioxidant function by improving enzymatic activity and reducing free radical damage. The absence of significant changes in other antioxidant parameters may reflect a balanced regulatory mechanism within the antioxidant system, wherein activation of one pathway may suppress others. The specific regulatory mechanisms warrant further investigation.

### **3.3 Effects of *Radix pseudostellariae* Stem and Leaf Polysaccharide on Serum Immune Indexes of Weaned Piglets**

Serum immunoglobulins are essential components of the humoral immune system. IgG provides antibacterial, antiviral, and antitoxin effects by neutralizing toxins; IgM is the primary antibody produced upon initial antigen exposure and also possesses antibacterial, antiviral, and antitoxin functions; and IgA plays a critical “barrier” role in local mucosal immunity of the respiratory and digestive tracts. Traditional Chinese medicine polysaccharides can counteract cyclophosphamide-induced suppression of antibody-forming cells, enhance B cell function, activate lymphocytes, and increase immunoglobulin levels. The present results demonstrate that RPSLP supplementation increased serum IgA, IgG, and IgM contents, suggesting that RPSLP may enhance humoral immune function by increasing lymphocyte numbers and conversion rates. Activated complement components play vital roles in immune regulation and defense against pathogen invasion. C3 and C4 are primarily involved in immune and non-immune pathway activation, attracting phagocytes and participating in foreign substance rejection. The significant increase in serum C3 and C4 contents indicates that RPSLP strengthens both immune and non-immune activation pathways, thereby enhancing overall immune function. These findings align with previous studies demonstrating that other traditional Chinese medicine polysaccharides can significantly increase serum C3 and C4 contents in normal and immunosuppressed animals.

### **3.4 Effects of *Radix pseudostellariae* Stem and Leaf Polysaccharide on Serum Biochemical Indexes of Weaned Piglets**

Serum TG content reflects lipid metabolism status, with elevated levels indicating metabolic disorders. The significant reduction in serum TG content across all treatment groups suggests that RPSLP promotes lipid metabolism, facilitating fat oxidation to provide energy for protein synthesis and thereby promoting

growth. Serum TP and ALB contents directly reflect protein absorption, synthesis, and catabolism, as well as immune status. Serum UN content indicates the efficiency of amino acid utilization for protein synthesis, with reduced UN levels reflecting enhanced protein anabolism. Previous studies have reported that lentinan and *Atractylodes macrocephala* polysaccharides can increase serum TP and ALB while decreasing UN content. The present results demonstrate that RPSLP supplementation increased serum TP and ALB contents and decreased UN content, indicating enhanced protein synthesis and growth promotion.

Serum ALP primarily originates from the liver, and elevated activity indicates hepatocellular degeneration or necrosis. ALT and AST are important aminotransferases in mitochondria, and their activity changes serve as sensitive indicators of liver and cardiac cell damage. CREA, mainly produced from muscle metabolism and excreted by the kidneys, is a primary indicator of renal function. The absence of significant effects on serum AST, ALT, and ALP activities or CREA content demonstrates the safety of RPSLP supplementation in weaned piglet diets.

## Conclusion

Dietary supplementation with *Radix pseudostellariae* stem and leaf polysaccharide can effectively improve average daily gain, reduce diarrhea rate, enhance immune function, and improve antioxidant capacity and serum biochemical profiles in weaned piglets. Under the conditions of this experiment, the appropriate supplemental level is 1,000 mg/kg.

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