Effects of Proanthocyanidins 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 proanthocyanidin supplementation on growth performance and serum antioxidant, immune, and biochemical indices in weaned piglets. One hundred twenty weaned piglets with an average age of 23 days were selected and randomly divided into 4 groups (control group and experimental groups I, II, and III), with 3 replicates per group and 10 piglets per replicate. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 50, 100, and 150 mg/kg proanthocyanidins, respectively. The experiment consisted of a 7-day preliminary period and a 30-day formal experimental period. The results showed: 1) Compared with the control group, the average daily gain of piglets in experimental groups I and II was significantly or extremely significantly increased ($P<0.05$ or $P<0.01$); the diarrhea rate of piglets in all experimental groups was extremely significantly decreased ($P<0.01$), with experimental group II having the lowest diarrhea rate. 2) Compared with the control group, the serum total antioxidant capacity of all experimental groups was significantly increased ($P<0.05$), with experimental group II being significantly higher than groups I and III ($P<0.05$); serum malondialdehyde content was significantly decreased and superoxide dismutase activity was significantly increased in all experimental groups ($P<0.05$), with no significant differences among the three groups ($P>0.05$). 3) Compared with the control group, serum immunoglobulin G and complement 3 contents in experimental group II were significantly increased ($P<0.05$); serum complement 4 content in all experimental groups was significantly increased ($P<0.05$), with no significant differences among the three experimental groups ($P>0.05$). 4) Compared with the control group, serum triglyceride and urea nitrogen contents in all experimental groups were significantly or extremely significantly decreased ($P<0.05$ or $P<0.01$), serum albumin content in experimental group II was extremely significantly increased ($P<0.01$),

and no significant differences were observed among groups for other serum biochemical indices (P>0.05). These results suggest that dietary proanthocyanidin supplementation in weaned piglets can increase daily gain, reduce diarrhea rate, improve serum antioxidant capacity and enhance immunity, with 100 mg/kg being the appropriate supplementation level.

Full Text

Effects of Proanthocyanidins on Growth Performance and Serum Antioxidant, Immune, and Biochemical Indices of Weaned Piglets

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Abstract

This trial was conducted to investigate the effects of dietary proanthocyanidins on growth performance and serum antioxidant, immune, and biochemical indices of weaned piglets. A total of 120 weaned piglets with an average age of 23 days were randomly assigned to four groups (control group and experimental groups I, II, and III) with three replicates per group and ten piglets per replicate. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 50, 100, and 150 mg/kg proanthocyanidins, respectively. The trial consisted of a 7-day adaptation period followed by a 30-day formal experimental period. The results showed that: (1) Compared with the control group, the average daily gain of piglets in experimental groups I and II was significantly or extremely significantly increased (P<0.05 or P<0.01), and the diarrhea rate in all experimental groups was extremely significantly decreased (P<0.01), with the lowest rate observed in experimental group II. (2) Serum total antioxidant capacity in all experimental groups was significantly higher than that in the control group (P<0.05), with experimental group II showing significantly higher values than groups I and III (P<0.05). Serum malondialdehyde content was significantly decreased and superoxide dismutase activity was significantly increased in all experimental groups (P<0.05), with no significant differences among the three experimental groups (P>0.05). (3) Serum immunoglobulin G and complement 3 contents in experimental group II were significantly increased compared with the control group (P<0.05). Serum complement 4 content was significantly increased in all experimental groups (P<0.05), with no significant differences among them (P>0.05). (4) Serum triglyceride and urea nitrogen contents were significantly or extremely significantly decreased in all experimental groups (P<0.05 or P<0.01), while serum albumin content in experimental group II was extremely significantly increased

(P<0.01). No significant differences were observed among groups for other serum biochemical indices (P>0.05). These results indicate that dietary proanthocyanidins can increase daily gain, reduce diarrhea rate, improve serum antioxidant capacity, and enhance immunity in weaned piglets, with 100 mg/kg being the optimal supplementation level.

Keywords: proanthocyanidins; weaned piglets; growth performance; antioxidant; serum biochemical indices

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Weaning stress in piglets has long been a major challenge in modern intensive pig production. The abrupt transition from sow' s milk to solid feed after weaning typically leads to reduced feed intake, slow growth, compromised immunity, and increased susceptibility to disease, resulting in a sharp rise in diarrhea incidence. To prevent disease and promote growth, producers often administer antibiotics through drinking water or feed to mitigate weaning stress. However, the widespread use of antibiotics has led to increasing drug resistance in pathogenic bacteria, along with growing concerns about drug residues in animal products and environmental pollution from agriculture, necessitating urgent solutions. As the country vigorously promotes the production of pollution-free agricultural products, research has focused on whether non-toxic, harmless substances extracted from natural plants can be added to diets to reduce weaning stress in piglets. Weaning generates large quantities of free radicals due to rapid growth. Proanthocyanidins, also known as procyanidins, are a class of polyphenolic compounds widely found in plants that can scavenge free radicals, alleviate oxidative stress, and improve growth rate. Proanthocyanidins consist of varying numbers of catechin or epicatechin units condensed together. The simplest forms are dimers of catechin, epicatechin, or catechin-epicatechin combinations, with trimers, tetramers, and up to decamers also existing. Based on polymerization degree, dimers to tetramers are typically classified as oligomers (OPC), while pentamers and above are called polymers (PPC). Among various proanthocyanidins, dimers are the most widely distributed and extensively studied. The structure of dimeric proanthocyanidins is shown in Figure 1 [Figure 1: see original paper]. Research indicates that proanthocyanidins possess strong antioxidant activity, can reduce oxidative damage, improve immunity, regulate digestive enzyme activity, and avoid problems associated with antibiotic abuse such as bacterial resistance and drug residues [1]. This study aimed to investigate the effects of dietary proanthocyanidins extracted from natural plants on growth performance, immunity, and antioxidant capacity in weaned piglets, thereby ameliorating weaning stress.

1.1 Experimental Materials

The proanthocyanidins used were extracted and purified from maritime pine bark, with a purity greater than 85%. The extraction process involved crushing the bark, mixing it with 95% ethanol, extracting at room temperature for 3

hours, filtering, concentrating under reduced pressure using a rotary vacuum evaporator, mixing with silica gel at a specific ratio, drying, and sealing for storage [2].

Laboratory equipment included: RT-9900 semi-automatic biochemical analyzer (Shenzhen Rayto Life Sciences Co., Ltd.), VIS-7200 visible spectrophotometer (Shanghai Meipuda 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.).

1.2 Experimental Animals and Design

One hundred and twenty healthy "Duroc × Landrace × Large White" crossbred piglets with an average age of 23 days, similar body weight, and balanced sex ratio (castrated males and females) were selected and randomly divided into four groups: control group and experimental groups I, II, and III. The control group received the basal diet, while experimental groups received the basal diet supplemented with 50, 100, and 150 mg/kg proanthocyanidins, respectively. Each group consisted of three replicates with ten piglets per replicate. All pigs had ad libitum access to pelleted feed and water throughout the trial and were managed according to routine farm procedures for deworming and vaccination. The trial included a 7-day adaptation period followed by a 30-day formal experimental period. The basal diet was formulated according to NRC (2012) nutrient requirements, with composition and nutrient levels shown in Table 1 .

1.3.1 Growth Performance Measurement

All experimental pigs were individually weighed after fasting at the beginning and end of the trial to calculate average daily gain (ADG). Daily feed intake was recorded for each group to calculate average daily feed intake (ADFI) and feed-to-gain ratio (F/G). Piglet behavior was observed daily, and diarrhea incidence was recorded to calculate diarrhea rate.

Diarrhea rate (%) = [(Number of diarrheic piglets × Days of diarrhea) / (Total number of piglets × Trial days)] × 100.

1.3.2 Blood Sample Collection and Processing

At the end of the trial, five piglets from each group were selected for blood collection. Blood samples (4-5 mL) were collected from the anterior vena cava into empty centrifuge tubes, centrifuged at 3,500 r/min for 10 minutes, and the serum was separated and stored at -20°C for subsequent analysis.

1.3.3 Immune Indices Determination

Serum immunoglobulin G (IgG), complement 3 (C3), and complement 4 (C4) contents were determined using immunoturbidimetry with assay kits purchased

from Zhejiang Yilikang Biotechnology Co., Ltd. Measurements were performed using the RT-9900 semi-automatic biochemical analyzer according to the kit instructions.

1.3.4 Biochemical Indices Determination

Serum triglyceride (TG), total protein (TP), albumin (ALB), creatinine (CR), 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 Clinical Laboratory of Fuzhou Air Force Hospital. Assay kits were purchased from Beijing Leadman Biochemistry Co., Ltd., and measurements were performed using the OLYMPUS AU2700 automatic chemical and immune analyzer.

1.3.5 Antioxidant Indices Determination

Serum superoxide dismutase (SOD) activity was measured using the xanthine oxidase method, malondialdehyde (MDA) content was determined using the thiobarbituric acid method, and total antioxidant capacity (T-AOC) was measured using the Fe3+ reduction method. Catalase (CAT) and glutathione peroxidase (GSH-Px) activities were determined using colorimetric methods. Assay kits were purchased from Nanjing Jiancheng Bioengineering Institute, and measurements were performed using the VIS-7200 visible spectrophotometer according to the kit instructions.

1.4 Statistical Analysis

Experimental data are expressed as mean ± standard deviation. One-way ANOVA was performed using SPSS 19.0 software, followed by multiple comparisons using LSD and Duncan' s methods. Differences were considered significant at $P<0.05$ and extremely significant at $P<0.01$.

2.1 Effects of Proanthocyanidins on Growth Performance of Weaned Piglets

As shown in Table 2 , compared with the control group, ADG in experimental groups I, II, and III increased by 17.24% ($P<0.05$), 17.87% ($P<0.01$), and 8.92% ($P>0.05$), respectively. Diarrhea rate in experimental groups I, II, and III decreased by 49.54% ($P<0.01$), 56.27% ($P<0.01$), and 52.29% ($P<0.01$), respectively, with no significant differences among the three experimental groups ($P>0.05$), though group II showed the lowest diarrhea rate. No significant differences were observed among groups for ADFI or F/G ($P>0.05$).

2.2 Effects of Proanthocyanidins on Serum Antioxidant Indices of Weaned Piglets

As shown in Table 3 , serum T-AOC in all experimental groups was significantly higher than that in the control group ($P<0.05$), but did not show a dose-dependent increase. Experimental group II exhibited significantly higher T-AOC than groups I and III ($P<0.05$). Serum MDA content in experimental groups I, II, and III decreased by 19.03% ($P<0.05$), 26.30% ($P<0.05$), and 21.11% ($P<0.05$), respectively, with no significant differences among experimental groups ($P>0.05$). Serum SOD activity in experimental groups I, II, and III increased by 22.86% ($P<0.01$), 26.32% ($P<0.01$), and 24.93% ($P<0.01$), respectively, with no significant differences among experimental groups ($P>0.05$). No significant differences were observed among groups for serum CAT or GSH-Px activity ($P>0.05$).

2.3 Effects of Proanthocyanidins on Serum Immune Indices of Weaned Piglets

As shown in Table 4 , dietary proanthocyanidins affected serum IgG, C3, and C4 contents in weaned piglets. Compared with the control group, serum IgG content in experimental groups I, II, and III increased by 10.88% ($P>0.05$), 24.49% ($P<0.05$), and 9.75% ($P>0.05$), respectively, with no significant differences among experimental groups ($P>0.05$). Serum C3 content increased by 4.17% ($P>0.05$), 14.58% ($P<0.05$), and 6.25% ($P>0.05$) in experimental groups I, II, and III, respectively, with experimental group II showing significant differences compared with groups I and III ($P<0.05$). Serum C4 content in experimental groups I, II, and III increased by 25% ($P<0.05$), 25% ($P<0.05$), and 50% ($P<0.05$), respectively, with no significant differences among experimental groups ($P>0.05$).

2.4 Effects of Proanthocyanidins on Serum Biochemical Indices of Weaned Piglets

As shown in Table 5 , with increasing proanthocyanidin supplementation, serum TG, UN, CR contents and ALT activity decreased, while serum TP, ALB contents and ALP, AST activity increased. Specifically, serum TG content in experimental groups I, II, and III decreased by 25.00% ($P<0.01$), 33.82% ($P<0.01$), and 30.88% ($P<0.01$), respectively, with no significant differences among experimental groups ($P>0.05$). Serum UN content decreased by 17.63% ($P<0.05$), 21.15% ($P<0.01$), and 28.53% ($P<0.01$), respectively, with no significant differences among experimental groups ($P>0.05$). Serum CR content decreased by 0.48%, 4.56%, and 8.13%, while ALT activity decreased by 2.69%, 7.74%, and 4.53%, respectively, but these differences were not significant ($P>0.05$). Serum ALB content increased by 7.93% ($P>0.05$), 19.39% ($P<0.01$), and 16.17% ($P<0.05$) in experimental groups I, II, and III, respectively. ALP activity increased by 5.08%, 8.07%, and 2.87%; TP content increased by 2.74%, 7.24%, and

3.01%; and AST activity increased by 5.44%, 2.17%, and 9.62%, respectively, but none of these differences reached statistical significance (P>0.05).

3.1 Effects of Proanthocyanidins on Growth Performance of Weaned Piglets

Proanthocyanidins demonstrated significant growth-promoting effects, resulting in uniform piglet size, good development, low disease incidence, and significantly reduced feed-to-gain ratio, thereby lowering production costs and improving economic efficiency [3]. Zhao et al. [4] reported that dietary proanthocyanidins supplementation, while not significantly affecting ADFI, ADG, or F/G in weaned piglets, could increase ADFI in stressed piglets and alleviate the decline in feed conversion efficiency. Yang et al. [5] found that grape proanthocyanidins significantly reduced feed intake and improved feed efficiency in 6-week-old broilers, significantly enhancing feed utilization during the later production period. The present results showing significantly improved ADG in weaned piglets supplemented with proanthocyanidins further confirm their growth-promoting effects. Additionally, the extremely significant reduction in diarrhea rate observed in experimental groups aligns with Mittal et al. [6], who reported that grape seed proanthocyanidins reduced post-weaning diarrhea in rats by decreasing intestinal permeability and improving antioxidant indices. The minimal effects of proanthocyanidins on feed-to-gain ratio and feed intake may be attributed to their light brown powder form and astringent taste [7], which could reduce diet palatability at high supplementation levels, preventing significant changes in feed intake.

3.2 Effects of Proanthocyanidins on Serum Antioxidant Capacity of Weaned Piglets

Serum antioxidant indices include SOD, GSH-Px, and CAT activities, as well as T-AOC, while MDA content is the most commonly used indicator of lipid peroxidation [8]. Early weaning induces a series of stress responses, including impaired intestinal function, reduced immunity and antioxidant capacity, decreased feed utilization, growth stagnation, and increased diarrhea, morbidity, and mortality rates. Proanthocyanidins possess strong antioxidant capacity. In this study, different doses of proanthocyanidins significantly increased serum T-AOC, which reflects the non-enzymatic antioxidant system and effectively reduces oxidative stress in weaned piglets. Serum SOD activity was extremely significantly increased in experimental groups; SOD can effectively scavenge excess free radicals, protecting weaned piglets from chronic oxidative damage and alleviating weaning weight loss. Serum CAT and GSH-Px activities showed non-significant improvements, but these enzymes can scavenge H2O2 and mitigate oxidative stress-induced damage. Furthermore, the series of antioxidant responses resulted in significantly reduced serum MDA content in experimental groups compared with the control, demonstrating that appropriate proanthocyanidin supplementation can enhance antioxidant capacity and protect tissues

from oxidative damage in weaned piglets.

Kim et al. [9] reported that proanthocyanidins could induce reduction in HepG2 cells damaged by tert-butyl hydroperoxide oxidation. Zhao et al. [4] demonstrated that dietary supplementation with 100 mg/kg proanthocyanidins significantly increased T-AOC in serum and liver of stressed piglets, improved serum SOD activity and anti-hydroxyl radical capacity, and enhanced serum GSH-Px activity. Peng et al. [10] found that grape seed powder significantly increased SOD and GSH-Px activities while decreasing MDA content in aged rats. The present results are consistent with these findings.

3.3 Effects of Proanthocyanidins on Serum Immune Indices of Weaned Piglets

The immune system of early-weaned piglets is not fully developed and only gradually becomes complete by 5 weeks of age. Consequently, piglets are highly susceptible to disease during the first two weeks post-weaning. Activation of the immune system increases free radical content [11], which can weaken immune function; therefore, dietary antioxidants that scavenge free radicals can enhance immunity. Additionally, flavonoids directly promote the function of various immune and inflammatory cells [12]. As a member of the bioflavonoid family, proanthocyanidins can enhance non-specific and humoral immune functions, increase corresponding immunoglobulin contents, and exert antibacterial, anti-inflammatory, and antiviral effects, providing some resistance to acute streptococcal infections commonly observed in stressed weaned piglets. This study demonstrated that dietary supplementation with 50, 100, and 150 mg/kg proanthocyanidins increased serum IgG, C3, and C4 contents, indicating that proanthocyanidins as a feed additive can enhance immunity in weaned piglets. These findings are similar to Yang [13], who reported that dietary proanthocyanidins increased B lymphocyte transformation rates, possibly because coccidia invasion damaged the intestinal mucosal barrier, allowing massive pathogen invasion that activated humoral immunity and promoted B lymphocyte antibody secretion, thereby preventing secondary diseases. Hao et al. [14] reported that dietary supplementation with 100 or 150 mg/kg sorghum proanthocyanidins significantly increased serum IgG, IgM, C3, C4, and interleukin-2 contents, enhanced both immunity and antioxidant capacity, and reduced weaning diarrhea rates, which aligns with our results.

3.4 Effects of Proanthocyanidins on Serum Biochemical Indices of Weaned Piglets

Proanthocyanidins possess strong antioxidant capacity that scavenges free radicals, protects tissues from oxidative damage, and enhances immunity. Their antibacterial, anti-inflammatory, and antiviral effects effectively reduce stress responses during the period when maternal antibodies and endogenous cellular and humoral immunity are low, alleviating losses from diarrhea, feed refusal, growth stagnation, and mortality, thereby maintaining weaned piglets in a sta-

ble and healthy state. This study found that with increasing proanthocyanidin supplementation, serum TP and ALB contents and ALP and AST activity increased, while serum TG, UN, CR contents and ALT activity decreased. These results are comparable to Ge et al. [15], who reported that pine needle extract significantly increased serum TP, ALB, and globulin contents and AST and ALT activities while significantly decreasing serum MDA content in piglets.

Conclusion

Dietary proanthocyanidins supplementation effectively enhances antioxidant capacity and immunity, reduces diarrhea rate, and improves growth performance to some extent in weaned piglets, with 100 mg/kg being the optimal supplementation level.

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