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

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Date: 2017-10-23T00:00:00+00:00

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. A total of 120 weaned piglets with an average age of 23 days were 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 ($P<0.05$); the 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, the serum immunoglobulin G and complement 3 contents in experimental group II were significantly increased ($P<0.05$); the 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, the serum triglyceride and urea nitrogen contents in all experimental groups were significantly or extremely significantly decreased ($P<0.05$ or $P<0.01$), the serum albumin content in experimental group II was extremely significantly increased ($P<0.01$), and

there were no significant differences in other serum biochemical indices among groups (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

Abstract

This trial was conducted to investigate the effects of dietary proanthocyanidins on growth performance and serum antioxidant, immune, and biochemical indices in 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 pre-feeding period followed by 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), 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 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), while 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), 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 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

Introduction

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 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 pollution of the agricultural ecological environment, necessitating urgent solutions. Currently, as the country vigorously promotes pollution-free agricultural products, research has focused on extracting non-toxic and harmless substances from natural plants for dietary supplementation 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 distributed 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 classified as 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 has demonstrated that proanthocyanidins possess exceptional antioxidant activity, can reduce oxidative damage, improve immune function, 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 supplementation with proanthocyanidins extracted from natural plants on growth performance, immunity, and antioxidant capacity in weaned piglets, thereby ameliorating weaning stress.

Materials and Methods

1.1 Experimental Materials

Proanthocyanidins 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 vacuum rotary evaporator, mixing with silica gel at a specific ratio, drying, and sealing for storage [2].

The following equipment was used: RT-9900 semi-automatic biochemical ana-

lyzer (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 constant temperature water bath (Guohua Electrical Appliance Co., Ltd.).

1.2 Experimental Animals and Design

A total of 120 healthy "Duroc × Landrace × Large White" crossbred piglets with an average age of 23 days and similar body weight, with balanced gender distribution (castrated males and females), were randomly divided into four groups (control group and experimental groups I, II, and III). The control group was fed the basal diet, while experimental groups I, II, and III were fed 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 standard farm procedures for deworming and vaccination. The trial included a 7-day pre-feeding period followed by a 30-day formal experimental period. The basal diet was formulated according to NRC (2012) nutrient requirements, and its composition and nutrient levels are shown in Table 1 .

1.3 Measurement Indices and Methods

1.3.1 Growth Performance Measurement All experimental pigs were individually weighed at the beginning and end of the trial after fasting to calculate average daily gain (ADG). Daily feed intake was recorded to calculate average daily feed intake (ADFI) and feed-to-gain ratio (F/G) for each group. 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 × Total 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 Index Determination Serum immunoglobulin G (IgG), complement 3 (C3), and complement 4 (C4) contents were determined using immunoturbidimetric assay kits purchased from Zhejiang Yikang Biotechnology Co., Ltd. Measurements were performed using an RT-9900 semi-automatic biochemical analyzer according to the kit instructions.

1.3.4 Biochemical Index 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 measured with assistance from the Clinical Laboratory of Fuzhou Air Force Hospital. Reagent kits were purchased from Beijing Leadman Biochemistry Co., Ltd., and measurements were performed using an OLYMPUS AU2700 automatic chemical and immune analyzer.

1.3.5 Antioxidant Index 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 assay kits purchased from Nanjing Jiancheng Bioengineering Institute. Measurements were performed using a 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, and multiple comparisons were conducted using LSD and Duncan' s methods. Differences were considered significant at $P<0.05$ and extremely significant at $P<0.01$.

Results

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 decreased by 49.54% ($P<0.01$), 56.27% ($P<0.01$), and 52.29% ($P<0.01$) in the three experimental groups, respectively, with no significant differences among them ($P>0.05$), though experimental 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 had significantly higher T-AOC than groups I and III ($P<0.05$). Serum MDA content decreased by 19.03% ($P<0.05$), 26.30% ($P<0.05$), and 21.11% ($P<0.05$) in experimental groups I, II, and III, respectively, with no significant differences among the three groups

(P>0.05). Serum SOD activity increased by 22.86% (P<0.01), 26.32% (P<0.01), and 24.93% (P<0.01) in the three experimental groups, respectively, with no significant differences among them (P>0.05). No significant differences were observed among groups for serum CAT or GSH-Px activities (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 increased by 10.88% (P>0.05), 24.49% (P<0.05), and 9.75% (P>0.05) in experimental groups I, II, and III, respectively, with no significant differences among the three 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), respectively, with experimental group II showing significant differences compared with groups I and III (P<0.05). Serum C4 content increased by 25% (P<0.05), 25% (P<0.05), and 50% (P<0.05) in the three experimental groups, respectively, with no significant differences among them (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 decreased by 25.00% (P<0.01), 33.82% (P<0.01), and 30.88% (P<0.01) in experimental groups I, II, and III, respectively, with no significant differences among 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 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 the three experimental groups, 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 these differences were not significant (P>0.05).

Discussion

3.1 Effects of Proanthocyanidins on Growth Performance of Weaned Piglets

Proanthocyanidins demonstrate significant growth-promoting effects, markedly increasing weight gain in weaned piglets. Experimental piglets showed uniform size, good growth and development, low disease incidence, and significantly reduced feed-to-gain ratio, thereby lowering production costs and improving

economic efficiency [3]. Although Zhao et al. [4] found that dietary proanthocyanidins did not significantly affect ADFI, ADG, or F/G in weaned piglets, they observed increased ADFI in stressed piglets and alleviation of feed conversion efficiency decline. Yang et al. [5] reported that proanthocyanidins significantly reduced feed intake and improved feed efficiency in 6-week-old broilers, markedly enhancing feed utilization during the later production period. The present study demonstrated that dietary proanthocyanidins significantly increased ADG in weaned piglets, further confirming their growth-promoting effects. Additionally, diarrhea rate was extremely significantly reduced in all experimental groups, consistent with Mittal et al. [6] who found that grape seed proanthocyanidins reduced diarrhea rate in weaned rats by decreasing intestinal permeability and improving antioxidant indices. The lack of significant effects on feed-to-gain ratio and feed intake may be attributed to the light brown color and astringent taste of proanthocyanidins [7], as high supplementation levels may reduce diet palatability and prevent 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 in piglets, including impaired intestinal function, reduced immunity and antioxidant capacity, decreased feed utilization, growth stagnation, and increased diarrhea, morbidity, and mortality rates. Proanthocyanidins possess exceptional antioxidant capacity. In this study, different doses of proanthocyanidins significantly increased serum T-AOC, which reflects the antioxidant capacity of the non-enzymatic 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 peroxidation damage and alleviating weaning weight loss. Serum CAT and GSH-Px activities showed non-significant improvements, yet they can scavenge H2O2 and mitigate oxidative stress-induced damage. Furthermore, following these antioxidant responses, serum MDA content was significantly reduced compared with the control group, indicating that appropriate dietary 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] found that dietary supplementation with 100 mg/kg proanthocyanidins significantly increased serum and liver T-AOC, improved serum SOD activity and anti-hydroxyl radical capacity, and enhanced serum GSH-Px activity in stressed piglets. Peng et al. [10] demonstrated that grape seed powder significantly increased SOD and GSH-Px activities while decreasing MDA content in aged rats. The present findings are consistent with these studies.

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 resistance against acute streptococcal infections commonly occurring 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 results are similar to Yang [13], who found that dietary proanthocyanidins increased B lymphocyte transformation rate, possibly because coccidia invasion destroyed intestinal mucosal barriers, causing massive pathogen invasion that activated humoral immunity and promoted B lymphocyte antibody secretion to prevent 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 immunity and antioxidant capacity, and reduced weaning diarrhea rate, which is also consistent with our findings.

3.4 Effects of Proanthocyanidins on Serum Biochemical Indices of Weaned Piglets

Proanthocyanidins possess strong antioxidant capacity, can scavenge free radicals, protect tissues from oxidative damage, and thereby improve immunity. Their antibacterial, anti-inflammatory, and antiviral effects effectively reduce stress responses in weaned piglets when maternal antibody levels and cellular and humoral immunity are low, alleviating losses from diarrhea, feed refusal, growth stagnation, and even death, thus maintaining piglets in a stable and healthy state. This study found that with increasing proanthocyanidin supplementation, serum TP and ALB contents and ALP and AST activities increased, while serum TG, UN, CR contents and ALT activity decreased. These results are similar to Ge et al. [15], who found that pine needle extract significantly increased serum TP, ALB, and globulin contents and AST and ALT activities while significantly reducing serum MDA content in piglets.

Conclusion

Dietary supplementation with proanthocyanidins can effectively improve antioxidant capacity and immunity, reduce diarrhea rate, and enhance growth perfor-

mance 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.