Effects of Chitosan on Growth Performance, Fecal Score, Serum Hormones, and T Lymphocyte Subsets in Weaned Piglets: Postprint

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Abstract

This study aimed to investigate the effects of chitosan on growth performance, fecal score, and serum hormones and T lymphocyte subsets in weaned piglets. Sixty 28-day-old weaned Duroc × Large White × Landrace crossbred piglets were selected and randomly divided into 5 groups (12 piglets per group): the control group was fed a basal diet, and the experimental groups were fed experimental diets supplemented with 250, 500, 1,000, and 2,000 mg/kg chitosan in the basal diet, respectively. The experimental period lasted 14 days. The results showed that: 1) dietary supplementation with 250–2,000 mg/kg chitosan significantly increased the average daily gain (ADG) of weaned piglets ($P<0.05$) and significantly decreased the feed-to-gain ratio (F/G) ($P<0.05$); 2) dietary supplementation with 250–2,000 mg/kg chitosan significantly decreased the fecal score of weaned piglets on day 11 of the experiment ($P<0.05$); 3) dietary supplementation with appropriate doses of chitosan significantly increased the concentrations of serum growth hormone-releasing hormone (GHRH) (250–2,000 mg/kg), growth hormone (GH) (500–1,000 mg/kg), and leptin (LP) (2,000 mg/kg) in weaned piglets ($P<0.05$), and significantly decreased the concentrations of serum corticotropin-releasing hormone (CRH) (250–2,000 mg/kg), adrenocorticotropic hormone (ACTH) (500–1,000 mg/kg), cortisol (COR) (250–2,000 mg/kg), and soluble CD8 (sCD8) (500–2,000 mg/kg) ($P<0.05$). These results indicate that dietary supplementation with appropriate doses of chitosan can promote growth, reduce diarrhea, and alleviate weaning stress in weaned piglets.

Full Text

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Abstract

This study aimed to investigate the effects of chitosan on growth performance, fecal score, serum hormones, and T lymphocyte subset of weaned piglets. Sixty Duroc × Large White × Landrace crossbred piglets weaned at 28 days of age were selected and randomly assigned to 5 groups (12 piglets per group). Piglets in the control group were fed a basal diet, while those in the experimental groups were fed the basal diet supplemented with 250, 500, 1,000, or 2,000 mg/kg chitosan. The experiment lasted for 14 days. The results showed that: 1) dietary supplementation with 250–2,000 mg/kg chitosan significantly increased average daily gain (ADG) ($P<0.05$) and significantly decreased feed-to-gain ratio (F/G) ($P<0.05$); 2) dietary supplementation with 250–2,000 mg/kg chitosan significantly decreased fecal score on day 11 ($P<0.05$); 3) dietary supplementation with appropriate doses of chitosan significantly increased serum concentrations of growth hormone-releasing hormone (GHRH) (250–2,000 mg/kg), growth hormone (GH) (500–1,000 mg/kg), and leptin (LP) (2,000 mg/kg) ($P<0.05$), and significantly decreased serum concentrations of corticotropin-releasing hormone (CRH) (250–2,000 mg/kg), adrenocorticotropic hormone (ACTH) (500–1,000 mg/kg), cortisol (COR) (250–2,000 mg/kg), and soluble CD8 (sCD8) (500–2,000 mg/kg) ($P<0.05$). These results suggest that dietary supplementation with appropriate doses of chitosan can promote growth, reduce diarrhea, and alleviate weaning stress in weaned piglets.

Keywords: chitosan; weaned piglets; growth performance; fecal score; T lymphocyte subset

Introduction

Weaning is the most significant stressor in the pig production cycle, involving separation of piglets from the sow and removal of maternal milk from their diet, which leads to disruption of the sow-piglet bond [1]. Weaned piglets may also be exposed to additional stressors, including changes in physical (housing, transportation) and social (unfamiliar pen-mates) environments [2]. These abrupt dietary and environmental changes often cause digestive dysfunction, gastrointestinal diseases, and diarrhea, resulting in growth retardation [3-4] and weaning stress. Consequently, many functional substances have been applied as growth promoters in weaning diets to control diarrhea in weaned animals [5-6].

Among these, chitosan, as a natural linear heteropolysaccharide, is a non-toxic nutritional supplement generally recognized as a safe compound [7]. Chitosan is derived from chitin, which is primarily found in the exoskeletons of crustaceans such as shrimp or crabs and in the cell walls of fungi. Chitosan is a copolymer of glucosamine [β-(1,4)-2-amino-2-deoxy-D-glucose] and N-acetylglucosamine (2-acetamido-2-deoxy-D-glucose) [8]. Recent studies have shown that chitosan possesses multiple biological functions, including antitumor [9-10], cholesterol-lowering [11], immune enhancement [12], antidiabetic [13], wound healing promotion [14], and antifungal and antimicrobial activities [15], and can improve growth performance in young animals [16]. This study aimed to investigate the effects of chitosan on growth performance, fecal score, and serum hormones and T lymphocyte subsets of weaned piglets, providing a scientific basis for the application of chitosan in the pig industry.

Materials and Methods

1.1 Test Materials

Chitosan: deacetylation degree of 85.09%, viscosity of 45 cps, purchased from Shandong Jinan Haidebei Marine Bioengineering Co., Ltd.

1.2 Experimental Design

Sixty healthy Duroc × Large White × Landrace crossbred weaned piglets [initial body weight of (8.85$±$1.52) kg] were selected and randomly divided into 5 groups, with 12 piglets per group, half male and half female. The five groups were fed experimental diets prepared by supplementing the basal diet with 0 (control group), 250, 500, 1,000, and 2,000 mg/kg chitosan, respectively. The basal diet was formulated as a complete powder feed according to the NRC (1998) nutrient requirements for swine, and its composition and nutrient levels are shown in Table 1 .

1.3 Husbandry Management

Experimental piglets were housed on netting beds [(2$×2.2)*m*^{2}]*innurseryrooms.Environmentalconditions*
days of age and transferred to nursery rooms, where they were fed a transitional diet for 7 days before the formal experiment began. During the formal experimental period, piglets were fed the experimental diets ad libitum with free access to water. The experimental period lasted for 14 days.

1.4 Measurement Indices

1.4.1 Growth Performance On the mornings of the first and last days of the experiment, piglets were weighed after fasting, and daily feed allowance and residual feed for each piglet were recorded to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

1.4.2 Fecal Score Based on the 5-point scale method described by Hu et al. [17], fecal morphology was subjectively scored by visual observation at 14:00 daily, rating from 1 to 5 (fecal scoring criteria are shown in Table 2), and fecal scores were recorded.

1.4.3 Serum Indices On the final morning of the experiment, 6 piglets with similar body condition were randomly selected from each group. Blood samples (10 mL) were collected from the anterior vena cava using vacuum tubes, left to stand for 20 min, then centrifuged at 1,500 r/min for 15 min. Serum was separated and stored at -20 °C for subsequent analysis. Serum concentrations of growth hormone-releasing hormone (GHRH), somatostatin (SS), growth hormone (GH), insulin-like growth factor-I (IGF-I), leptin (LP), corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), cortisol (COR), and T lymphocyte subsets including soluble CD3 (sCD3), soluble CD4 (sCD4), and soluble CD8 (sCD8) were measured according to kit instructions. CRH and GHRH assay kits were purchased from Nanjing Boerdi Biotechnology Co., Ltd., while kits for other indices were purchased from Nanjing Jiancheng Bioengineering Institute.

1.5 Statistical Analysis

Experimental data were organized using Excel 2007 and subjected to regression analysis using SAS 9.0 statistical software, with Duncan' s multiple comparison test performed (except for fecal score). Fecal score data were analyzed using two-factor ANOVA to examine main effects (time and diet) and their interaction. $P<0.05$ was considered statistically significant.

Results

2.1 Effects of Chitosan on Growth Performance of Weaned Piglets

As shown in Table 3 , with increasing chitosan supplementation levels, ADG of weaned piglets showed significant linear and quadratic effects ($P<0.05$), while F/G showed significant linear and quadratic decreasing effects ($P<0.05$). ADG in all chitosan-supplemented groups was significantly higher than that in the control group ($P<0.05$), and F/G was significantly lower than that in the control group ($P<0.05$). However, dietary chitosan supplementation had no significant effect on final body weight or ADFI of weaned piglets ($P>0.05$).

2.2 Effects of Chitosan on Fecal Score of Weaned Piglets

As shown in Figure 1 [Figure 1: see original paper], on day 2, fecal scores in the 500 and 2,000 mg/kg chitosan groups were significantly lower than in the control group (P<0.05); on day 3, fecal scores in the 250 and 2,000 mg/kg chitosan groups were significantly lower than in the control group (P<0.05); on days 5, 10, and 12, fecal scores in the 1,000 mg/kg chitosan group were significantly lower than in the control group (P<0.05); and on day 11, fecal scores in all chitosan-supplemented groups were significantly lower than in the control group (P<0.05). Throughout the experimental period, both diet and time had significant effects on fecal score of weaned piglets (P<0.05), but there was no significant interaction between diet and time (P>0.05).

2.3 Effects of Chitosan on Serum Growth Axis Hormone Concentrations of Weaned Piglets

As shown in Table 4 , with increasing chitosan supplementation levels, serum GHRH and GH concentrations showed significant quadratic increasing effects (P<0.05), while serum LP concentration showed a significant linear increasing effect (P<0.05). Serum GHRH concentrations in all chitosan-supplemented groups were significantly higher than in the control group (P<0.05). Serum GH concentrations in the 500 and 1,000 mg/kg chitosan groups were significantly higher than in the control group (P<0.05), and serum LP concentration in the 2,000 mg/kg chitosan group was significantly higher than in the control group (P<0.05).

2.4 Effects of Chitosan on Serum Stress Hormone Concentrations of Weaned Piglets

As shown in Table 5 , with increasing chitosan supplementation levels, serum CRH concentration showed a quadratic decreasing trend (P=0.088), and serum CRH concentrations in all chitosan-supplemented groups were significantly lower than in the control group (P<0.05). Serum ACTH and COR concentrations showed significant quadratic decreasing effects (P<0.05). Serum ACTH concentrations in the 500 and 1,000 mg/kg chitosan groups were significantly lower than in the control group (P<0.05), and serum COR concentrations in all chitosan-supplemented groups were significantly lower than in the control group (P<0.05).

2.5 Effects of Chitosan on Serum T Lymphocyte Subset of Weaned Piglets

As shown in Table 6 , with increasing chitosan supplementation levels, serum sCD8 concentration showed a significant quadratic decreasing effect (P<0.05), and serum sCD8 concentrations in the 500, 1,000, and 2,000 mg/kg chitosan groups were significantly lower than in the control group (P<0.05). No significant linear or quadratic effects were observed for serum sCD3, sCD4 concentrations, or sCD4/sCD8 ratio (P>0.05).

Discussion

When animals are exposed to environmental, nutritional, and immune stressors, various metabolic processes are negatively affected, leading to digestive disorders, diarrhea, reduced growth performance, and increased mortality. Particularly during the weaning period, piglets have immature digestive and immune systems, making diarrhea accompanied by dehydration and even death common. Previous studies have shown that chitosan, as a natural alkaline polysaccharide, possesses growth-promoting properties in young animals. Liu et al. [18] reported that dietary supplementation with 0.01% or 0.02% chitosan positively affected feed intake, body weight gain, and feed efficiency in weaned piglets. Zhou et al. [19] evaluated the effects of chitosan supplementation on growth performance, nutrient digestibility, and diarrhea incidence in weaned pigs, demonstrating that 0.20% chitosan improved growth performance, enhanced apparent total tract digestibility of dry matter and nitrogen, and reduced diarrhea incidence. The present study showed that dietary chitosan supplementation improved growth performance in weaned piglets, possibly due to increased serum concentrations of GHRH and GH. Tang et al. [20] demonstrated that chitosan increased plasma GH and IGF-I concentrations and IGF-I mRNA abundance in liver and muscle of weaned piglets, suggesting that dietary chitosan supplementation may improve growth performance and feed conversion efficiency by increasing plasma GH and IGF-I concentrations, which is consistent with our findings.

Additionally, this study found that dietary supplementation with 2,000 mg/kg chitosan significantly increased serum LP concentration in weaned piglets. LP is a protein hormone secreted by white adipose tissue that participates in the regulation of lipid metabolism [21]. Numerous studies have indicated that LP acts on central brain signals to inhibit feed intake, increase energy expenditure, and suppress lipogenesis while promoting lipolysis [22-23]. In this study, dietary supplementation with 2,000 mg/kg chitosan significantly increased serum LP concentration but did not negatively affect growth performance. This may be because weaned piglets primarily undergo skeletal development and muscle deposition rather than fat deposition, and the increased serum LP concentration did not substantially affect growth performance.

The weaning period is one of the most rapid phases of immunological and behavioral changes in piglets. During this time, piglets undergo physiological changes in intestinal structure and function (enzyme activity, absorption, and secretion) [3,24-25]. These alterations affect the digestive, absorptive, and secretory capacity of the small intestine and can adversely impact intestinal barrier function [24,26-28]. When the intestinal mucosal barrier is compromised, intestinal epithelial permeability increases, allowing toxins, bacteria, or feed-related antigens to cross the intestinal epithelium, leading to inflammation, malabsorption, diarrhea, and reduced growth performance. Among these, Escherichia coli is considered one of the most important causes of post-weaning diarrhea in piglets. Therefore, reduction of E. coli populations can decrease diarrhea incidence in weaned piglets [29]. In this study, chitosan-supplemented groups showed lower fecal scores compared with the control group. Combined with our previous research [30], chitosan supplementation reduced intestinal E. coli populations in weaned piglets. Liu et al. [18] also found that supplementation with 0.01% or 0.02% chitosan reduced fecal E. coli counts, increased Lactobacillus populations, and decreased diarrhea incidence. Furthermore, chitosan can bind certain types of bacteria and may interfere with their adhesion to intestinal tissues of the host animal [18,31], reducing intestinal mucosal damage. Chitosan can also delay the passage of digesta through the intestine and has water-absorbing capacity [32], thereby reducing fecal scores and diarrhea incidence.

After weaning, increased serum CRH and COR concentrations indicate activation of pathways mediating CRH receptor-induced intestinal dysfunction [33]. The present study showed that dietary chitosan supplementation decreased serum CRH, ACTH, and COR concentrations in weaned piglets. CRH regulates gastrointestinal function through activation of the central hypothalamic-pituitary-adrenal axis or peripheral CRH-based paracrine systems [34]. Abnormal release of neuroendocrine factors such as CRH, ACTH, and COR can cause intestinal cytokine imbalance, impair intestinal barrier function, and increase intestinal epithelial permeability [35-36], allowing bacteria and antigens to cross the epithelial barrier and trigger inflammation and diarrhea. Chen et al. [37] also found that chitosan decreased blood COR concentration in immunologically stressed piglets. These findings suggest that chitosan can alleviate weaning stress and protect intestinal barrier integrity, thereby reducing diarrhea. Moreover, CRH is produced by parvocellular neurons in the paraventricular nucleus of the hypothalamus and binds to CRH receptors in the anterior pituitary to induce ACTH release, which subsequently stimulates adrenal glucocorticoid release [38]. Glucocorticoids are important regulators of inflammation and immune responses, and through these stress hormones, stress adversely affects immune function, including reduced natural killer (NK) cell activity and lymphocyte subsets and proliferation [39], leading to decreased immunity and increased disease susceptibility. In this study, dietary chitosan supplementation decreased serum CRH, ACTH, and COR concentrations in weaned piglets, reflecting the stress-alleviating effect of chitosan and potentially having a positive impact on immune function.

Cellular immune responses play an important role in host defense against intracellular pathogens by inhibiting pathogen replication and accelerating clearance of infected cells. Soluble CD3 (sCD3), sCD4, and sCD8 in peripheral blood are soluble forms of CD3+, CD4+, and CD8+, which are associated with T lymphocyte activation [40]. These soluble forms have been identified as important markers of T lymphocyte activation and disease or infection occurrence. In this study, dietary chitosan supplementation decreased serum sCD8 concentration in weaned piglets, indicating that chitosan can modulate T lymphocyte immune function. Our previous research demonstrated that chitosan has inhibitory effects on lymphocyte function [41]. Therefore, immune function suppression may be one pathway through which chitosan improves growth performance, as immune activation is accompanied by altered metabolic activity that leads to nutrient reprioritization to support defense against foreign antigens [42]. Furthermore, this study and our previous research [43] showed that chitosan can improve growth performance in weaned piglets, which may reflect improved immune function.

Overall, dietary supplementation with different doses of chitosan can promote growth, reduce diarrhea incidence, alleviate stress, and enhance cellular immunity in weaned piglets to varying degrees. However, based on the experimental values, the most pronounced effects were observed with 500 mg/kg chitosan supplementation, with diminishing effects at higher supplementation levels. This may be because chitosan, as a positively charged mucopolysaccharide that is difficult for mammalian digestive enzymes to digest, can increase intestinal content viscosity [11]. Additionally, its anion-exchange properties can affect bile acid circulation and increase fat excretion. High doses of chitosan may interfere with the digestion and absorption of nutrients, particularly fats and fat-soluble vitamins, causing nutrient reprioritization within the body. Therefore, the regulatory effects of chitosan on growth and immunity in weaned piglets exhibit dose-dependent effects.

Conclusions

1. Dietary chitosan supplementation improved growth performance and increased serum GHRH and GH concentrations in weaned piglets, while high-dose chitosan (2,000 mg/kg) increased serum LP concentration.

2. Dietary chitosan supplementation decreased fecal scores and reduced serum CRH, ACTH, COR, and sCD8 concentrations in weaned piglets.

3. In conclusion, dietary supplementation with appropriate doses of chitosan can promote growth, reduce diarrhea, and alleviate weaning stress in weaned piglets.

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