Effects of Chitosan Oligosaccharide on Immunity and Related Physicochemical Indicators in Weaned Piglets: Postprint

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Abstract

This experiment aimed to investigate the effects of chitosan oligosaccharide on immunity and related physicochemical indices in weaned piglets. A total of 256 21-day-old "Duroc × Landrace × Yorkshire" crossbred weaned piglets were selected and randomly divided into 4 groups, with 4 replicates per group and 16 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 g/t chitosan oligosaccharide, respectively. The pre-trial period was 7 days, and the experimental period was 28 days. The results showed that: 1) Compared with the control group, serum aspartate aminotransferase activity in experimental groups 1, 2, and 3 decreased by 26.71% ($P<0.01$), 7.56% ($P>0.05$), and 16.64% ($P<0.05$), respectively; serum alanine aminotransferase activity decreased by 47.26% ($P<0.05$), 47.28% ($P<0.01$), and 21.88 ($P>0.05$), respectively; serum alkaline phosphatase activity increased by 6.37%, 16.78%, and 1.24% ($P>0.05$), respectively; serum total protein content in all experimental groups was higher than that in the control group ($P>0.05$). 2) Compared with the control group, serum immunoglobulin A content in experimental groups 1, 2, and 3 increased by 45.40% ($P<0.05$), 2.62% ($P>0.05$), and 137.26% ($P<0.01$), respectively; serum immunoglobulin G content in experimental groups 1 and 2 increased by 4.47% and 10.92% ($P>0.05$), respectively, compared with the control group, and serum IgM content increased by 9.29% and 5.69% ($P>0.05$), respectively; serum tumor necrosis factor-α content in experimental groups 1, 2, and 3 increased by 51.76% ($P<0.01$), 29.42% ($P>0.05$), and 88.29% ($P<0.01$), respectively, compared with the control group. 3) Compared with the control group, serum antibody titers against pseudorabies, classical swine fever, and porcine reproductive and respiratory syndrome were improved to varying degrees in the experimental groups, with serum pseudorabies and porcine reproductive and respiratory syndrome antibody titers in experimental group 3 being significantly higher than those in the control group (P<0.05). It can be concluded that dietary supplementation with appropriate chitosan oligosaccharide can improve humoral and cellular immune capacity and serum antibody titers in weaned piglets to a certain extent, and alleviate weaning stress.

Full Text

Effects of Chitosan Oligosaccharide on Immunity and Related Physiological and Biochemical Indices of Weaned Piglets

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Abstract: This experiment was conducted to investigate the effects of chitosan oligosaccharide on immunity and related physiological and biochemical indices in weaned piglets. A total of 256 "Duroc × Landrace × Yorkshire" crossbred piglets weaned at 21 days of age were randomly allocated into 4 groups with 4 replicates per group and 16 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 g/t chitosan oligosaccharide, respectively. The experiment consisted of a 7-day pre-trial period followed by a 28-day formal trial period. The results showed: 1) Compared with the control group, serum aspartate aminotransferase (AST) activity in experimental groups 1, 2, and 3 decreased by 26.71% (P<0.01), 7.56% (P>0.05), and 16.64% (P<0.05), respectively; serum alanine aminotransferase (ALT) activity decreased by 47.26% (P<0.05), 47.28% (P<0.01), and 21.88% (P>0.05), respectively; serum alkaline phosphatase (ALP) activity increased by 6.37%, 16.78%, and 1.24% (P>0.05), respectively; and serum total protein content in all experimental groups was higher than that in the control group (P>0.05). 2) Compared with the control group, serum immunoglobulin A (IgA) content in experimental groups 1, 2, and 3 increased by 45.40% (P<0.05), 2.62% (P>0.05), and 137.26% (P<0.01), respectively; serum immunoglobulin G (IgG) content in experimental groups 1 and 2 increased by 4.47% and 10.92% (P>0.05), respectively; serum IgM content increased by 9.29% and 5.69% (P>0.05), respectively; and serum tumor necrosis factor-α (TNF-α) content increased by 51.76% (P<0.01), 29.42% (P>0.05), and 88.29% (P<0.01), respectively. 3) Compared with the control group, serum antibody titers against pseudorabies virus, classical swine fever virus, and porcine reproductive and respiratory syndrome virus were all improved to varying degrees, with experimental group 3 showing significantly higher pseudorabies and PRRS antibody titers (P<0.05). These results indicate that dietary supplementation with appropriate levels of chitosan oligosaccharide can enhance humoral and cellular immunity, improve serum antibody titers, and alleviate weaning stress in weaned piglets.

Keywords: chitosan oligosaccharide; weaned piglets; physiological and bio-

chemical indices; immunity

Antibiotics have been used as feed additives for several decades, and their inclusion in feed has become a common practice to improve production efficiency. However, as antibiotic use has become more widespread, problems have gradually emerged. The misuse of antibiotics can cause dual infections, and bacterial resistance is making common diseases increasingly difficult to cure. Antibiotic residues in animal products can cause poisoning or allergic reactions in consumers, while residual antibiotics discharged into the environment pollute water and soil, disrupting ecological balance.

To reduce antibiotic usage, the development and screening of antibiotic alternatives has become a research hotspot. Oligosaccharides are one such class of products that have gradually attracted attention. Studies have confirmed that chitosan oligosaccharide can reduce diarrhea incidence in livestock and poultry, alleviate infection-related phenomena, and positively affect animal growth performance as well as apparent digestibility of dry matter and nitrogen [1-2]. This experiment was designed to investigate the effects of chitosan oligosaccharide on cellular immunity, humoral immunity, and antibody titers in weaned piglets, thereby understanding its impact on physiological indices and immunity, and to establish application protocols for chitosan oligosaccharide in piglet diets.

1.1 Experimental Material

Chitosan oligosaccharide was provided by Qinhuangdao Leader Bio-Agriculture Co., Ltd., with an active ingredient content of 75%, dark brown color, average molecular weight $ $2,500 u, and degree of deacetylation $ $90%.

1.2 Experimental Design and Animals

A total of 256 healthy "Duroc × Landrace × Yorkshire" crossbred piglets weaned at 21 days of age, with similar body weight and identical breed, were randomly divided into 4 groups according to the principles of identical genetic background and consistent sex ratio. Each group had 4 replicates with 16 piglets per replicate, and there was no significant difference in initial body weight among groups ($P>0.05$). The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 50, 100, and 150 g/t chitosan oligosaccharide, respectively. The experiment included a 7-day pre-trial period and a 28-day formal trial period.

1.3 Experimental Diets

Piglets were fed a corn-soybean meal type diet without antibiotics. The diet was formulated according to NRC (2012) and China' s "Feeding Standard of Swine" (NY/T 65—2004). The composition and nutrient levels of the basal diet are shown in Table 1 .

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

Items	Content
Ingredients	
Corn	
Wheat middling	
Soybean oil	
Soybean meal	
Soy protein isolate	
Extruded soybean	
Corn protein meal	
Import fish meal	
Whey powder	
Glucose	
NaCl	
Limestone	
CaHPO4	
Acidifier	
Premix[1])	
Total	
Nutrient levels[2])	
DE/(MJ/kg)	
CP	
Ca	
TP	
AP	
Lys	
Met	
Thr	

[1]) Premix provided the following per kg of the diet: VA 5,175 IU, VD_3 1,150 IU, VE 11.5 IU, VK_3 1.15 mg, VB_1 0.575 mg, VB_2 3.45 mg, VB_6 0.23 mg, VB_{12} 14.5 µg, riboflavin 3.45 mg, nicotinic acid 11.5 mg, pantothenic acid 5.75 mg, biotin 11.5 µg, Fe (as ferrous sulfate) 75 mg, Cu (as copper sulfate) 125 mg, Mn (as manganese sulfate) 20 mg, I (as potassium iodide) 0.5 mg, Se (as sodium selenite) 0.175 mg.
[2]) DE was a calculated value, while the others were measured values.

1.4 Animal Management

The experiment was conducted in a closed pig house at Hebei Han-Tang Animal Husbandry Co., Ltd. Experimental piglets were housed in separate pens under suitable temperature and humidity conditions. Piglets had free access to feed and water, received routine disinfection and vaccination, and were managed by designated personnel according to the standard feeding procedures for this breed.

1.5 Sample Collection and Processing

Three days before the end of the experiment, 8 piglets were randomly selected from each group. Blood (20 mL) was collected from the jugular vein, with 10 mL placed in heparin anticoagulant tubes and the remaining 10 mL in ordinary centrifuge tubes. After standing at low temperature for 30 minutes, the samples were centrifuged at 3,000 r/min for 15 minutes to collect serum, which was stored at -20°C for subsequent analysis.

1.6 Measurement Indices and Methods

Serum antibodies: Porcine enzyme-linked immunosorbent assay (ELISA) kits were used to determine serum immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) contents, as well as antibody titers against classical swine fever virus, pseudorabies virus, and porcine reproductive and respiratory syndrome virus. All measurements were performed strictly according to the kit instructions.

Cellular immunity: Serum interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) contents were determined using ELISA kits according to the manufacturer' s instructions.

Whole blood routine physiological indices: White blood cell count, red blood cell count, hemoglobin content, and platelet count were measured using a BC-2800Vet automatic blood analyzer (Mindray Medical International Limited).

Serum biochemical indices: Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT, alanine substrate method), creatine phosphokinase (CPK), and alkaline phosphatase (ALP) activities, as well as urea nitrogen (UN), total protein (TP), albumin (ALB), glucose (GLU), total bilirubin (TBIL), creatinine (CREA), total cholesterol (CHOL), and triglyceride (TG) contents were determined using a Microlab300 semi-automatic biochemical analyzer (Vital Scientific, Netherlands) with reagent kits purchased from Beijing Zhongkong Biotech Co., Ltd. Serum AST activity was measured by the aspartate substrate method, ALT activity by the alanine substrate method, and ALP activity by the NPP substrate-AMP buffer method. Serum TP content was determined by the biuret method, and ALB content by the bromocresol green method.

1.7 Statistical Analysis

Data were analyzed using the ANOVA model in SPSS 19.0 statistical software for one-way analysis of variance, with LSD method used for multiple comparisons.

2.1 Effects of Dietary Chitosan Oligosaccharide on Immunity of Weaned Piglets

As shown in Table 2 , compared with the control group, serum IgA content in experimental groups 1, 2, and 3 increased by 45.40% (P<0.05), 2.62% (P>0.05), and 137.26% (P<0.01), respectively. Serum IgG content in experimental groups 1 and 2 increased by 4.47% and 10.92% (P>0.05), respectively, while serum IgM content increased by 9.29% and 5.69% (P>0.05), respectively. Serum TNF-α content in experimental groups 1, 2, and 3 increased by 51.76% (P<0.01), 29.42% (P>0.05), and 88.29% (P<0.01), respectively, compared with the control group. Serum IL-6 content in experimental group 2 increased by 14.28% (P>0.05) compared with the control group.

Compared with the control group, serum pseudorabies antibody titers in all experimental groups showed varying degrees of improvement, with experimental group 3 showing a 46.51% increase (P<0.05). Classical swine fever antibody titers in experimental groups 1, 2, and 3 were higher than those in the control group by 5.40%, 5.40%, and 18.92% (P>0.05), respectively. Porcine reproductive and respiratory syndrome antibody titers in experimental groups 1, 2, and 3 increased by 17.65% (P>0.05), 29.41% (P>0.05), and 47.06% (P<0.05), respectively, compared with the control group.

Table 2 Effects of dietary chitosan oligosaccharide on immunity of weaned piglets

<table>
<tr><th>Items</th><th>Control group</th><th>Experimental group 1</th><th>Experimental group 2</th><th>Experimental group 3</th></tr>
<tr><td>IgA (μg/mL)</td><td>29.01$±5.07Ad [illegible] (ng/L)</td><td colspan="3">[illegible] 6(ng/L)|22.40±6.37Bbc|18.61±4.29ABb|25.60±7.46Bc|11.24±8.56Aa||PRV−Ab|0.43±0.23a|0.43±0.14a|0.44±0.15a|0.63±0.15b||CSF−Ab|0.37±0.11|0.39±0.07|0.39±0.06|0.44±0.07||PRRS−Ab|0.17±0.06a|0.20±0.06ab|0.22±0.05ab|0.25±$0.05b</td></tr>
</table>

In the same row, values with no letter or the same small letter superscripts mean no significant difference (P>0.05), while with different small letter superscripts mean significant difference (P<0.05), and with different capital letter superscripts mean extremely significant difference (P<0.01). The same as below.

2.2 Effects of Dietary Chitosan Oligosaccharide on Blood Indices of Weaned Piglets

As shown in Table 3 , dietary chitosan oligosaccharide supplementation had no significant effect on white blood cell count, red blood cell count, platelet count, or hemoglobin content in weaned piglets (P>0.05), and there were no significant differences among experimental groups (P>0.05). Compared with the control group, white blood cell counts in experimental groups 1, 2, and 3 decreased by 16.45%, 16.71%, and 16.29%, respectively, but the differences were not significant (P>0.05). Red blood cell counts in experimental groups 2 and 3 increased by 26.65% and 1.74%, respectively, while those in experimental group 1 decreased by 8.04% compared with the control group. Platelet counts in all experimental groups were higher than those in the control group to varying degrees, but the differences were not significant (P>0.05).

Compared with the control group, serum AST activity in experimental groups 1, 2, and 3 decreased by 26.71% (P<0.01), 7.56% (P>0.05), and 16.64% (P<0.05), respectively; serum ALT activity decreased by 47.26% (P<0.05), 47.28% (P<0.01), and 21.88% (P>0.05), respectively; serum ALP activity increased by 6.37%, 16.78%, and 1.24% (P>0.05), respectively. Serum CREA content in experimental groups 2 and 3 increased by 12.25% and 26.41% (P>0.05), respectively. Serum TG content in experimental groups 1 and 3 increased by 25.84% and 5.62% (P>0.05), respectively. Serum TP, ALB, and GLB contents in all experimental groups were higher than those in the control group to varying degrees, but the differences were not significant (P>0.05).

Table 3 Effects of dietary chitosan oligosaccharide on blood indices of weaned piglets

Items	Control group	Experimental group 1	Experimental group 2	Experimental group 3
WBC ($\times 10^{9}/L$)	31.00±4.52	25.90±5.01	25.82±5.69	25.95±7.00
RBC ($\times 10^{12}/g$)	6.34±0.61	5.83±0.42	8.03±3.37	6.45±0.21

3.1 Effects of Dietary Chitosan Oligosaccharide on Immunity of Weaned Piglets

Immunoglobulins are important immune effector molecules. IgG is the most abundant immunoglobulin in serum and the primary immunoglobulin in the body, and its content reflects the immune status of the organism. IgM is the immunoglobulin with the largest molecular weight and is the first immunoglobulin produced during the primary immune response. IL-6 and TNF-α are two important cytokines secreted by activated macrophages. IL-6 can promote lymphocyte proliferation and is involved in the activation of T lymphocytes, cytotoxic T cells (CTL), natural killer (NK) cells, and lymphokine-activated killer (LAK) cells. While enhancing immunity, IL-6 and TNF-α can also promote inflammatory responses and are cytokines with both anti-inflammatory and pro-inflammatory effects. Serum IL-6 and interleukin-1β (IL-1β) contents are the most representative indicators of inflammatory response during stress [3].

Studies have found that oligosaccharide supplementation can regulate serum immunoglobulin content [4-5]. Huang et al. [6] investigated the effects of chitosan oligosaccharide on broilers and found that the number of immunoglobulins in blood significantly increased in the chitosan oligosaccharide group, indicating that chitosan oligosaccharide can enhance broiler immunity. Tang et al. [7] reported that dietary supplementation with 250 mg/kg chitosan oligosaccharide increased blood IL-1, IL-2, and IL-6 contents, as well as IgA, IgM, and IgG contents. Chitosan oligosaccharide also affects serum immunoglobulins in piglets; dietary supplementation with 0.01%-0.05% chitosan can increase serum immunoglobulin content in a dose-dependent manner [8-9], with similar findings reported by Yin et al. [10]. The present study showed that dietary chitosan oligosaccharide supplementation significantly increased serum IgA and TNF-α contents, consistent with the above reports. However, the results for serum IgG, IgM, and IL-6 were not consistent with previous reports, possibly due to differences in rearing environment, animal species, and chitosan oligosaccharide supplementation levels.

Mannanase supplementation can improve both cellular and humoral immune functions. After piglets are vaccinated against classical swine fever and pseudorabies, B lymphocytes in the body recognize, activate, proliferate, and differentiate into plasma cells that secrete antibodies, which then enter the bloodstream. Li et al. [11] reported that mannan oligosaccharide could increase classical swine fever antibody titers, possibly because oligosaccharides can competitively adsorb and exclude pathogenic bacteria and specifically bind toxins, acting as adjuvants for these foreign antigens and slowing antigen absorption, thereby enhancing antigen efficacy [12]. The present study confirmed that chitosan oligosaccharide supplementation improved pseudorabies, classical swine fever, and PRRS antibody titers in weaned piglets to varying degrees.

3.2 Effects of Dietary Chitosan Oligosaccharide on Whole Blood Physiological Indices of Weaned Piglets

In this study, white blood cell counts in all chitosan oligosaccharide-supplemented groups showed a decreasing trend compared with the control group. White blood cells directly participate in cell-mediated immune responses and phagocytosis [13]. Lin et al. [14] added 2,000 mg/kg chitosan oligosaccharide to the basal diet of koi carp and found that total white blood cell counts in serum significantly increased. Su et al. [15] added chitosan oligosaccharide to the diet of tiger puffer and found no significant effect on white blood cell counts in serum. The present study found that dietary chitosan oligosaccharide supplementation showed a decreasing trend in white blood cell counts in weaned piglets, though not significant. This differs from other studies, and the specific reasons require further investigation.

3.3 Effects of Dietary Chitosan Oligosaccharide on Serum Biochemical Indices of Weaned Piglets

Serum biochemical indices are closely related to metabolism, nutritional status, and disease in animals, and can reflect physiological and pathological changes to some extent [16]. Huo et al. [17] reported that dietary chitosan oligosaccharide supplementation had no significant effect on blood AST and ALT activities in juvenile turbot, though the 200 mg/kg chitosan oligosaccharide group showed significantly lower enzyme activities than the control group. Liu et al. [18] found that chitosan oligosaccharide decreased serum AST and ALT activities in rainbow trout, but the differences were not significant. AST and ALT mainly exist in myocardial and liver cells, and their serum levels increase only when these cells are damaged. Studies have confirmed that appropriate chitosan oligosaccharide supplementation has protective effects on the liver and myocardium of piglets [19-20]. In this study, the decreased AST and ALT activities were consistent with previous reports, indicating that chitosan oligosaccharide has a regulatory effect on liver function.

Serum TP content can also reflect the nutritional and immune status of the organism [21]. Increased TP content indicates vigorous protein metabolism, which is beneficial for protein absorption and utilization, thereby reducing feed consumption [22]. Sun et al. [23] reported that dietary chitosan oligosaccharide supplementation could significantly decrease serum total cholesterol and low-density lipoprotein cholesterol contents in juvenile GIFT tilapia. Tang et al. [7] studied the effects of chitosan oligosaccharide on piglets and found that dietary oligosaccharide supplementation could decrease serum total cholesterol and TG contents and reduce the negative effects of weaning on serum antibody levels. Li et al. [24] investigated the effects of chitosan oligosaccharide on ducks and found that as a dietary supplement, chitosan oligosaccharide could reduce blood lipids in broilers by decreasing serum TG and total cholesterol contents. Maintaining blood lipids at lower levels helps prevent hyperlipidemia and improves immunity to some extent.

In this study, different levels of chitosan oligosaccharide supplementation increased serum TP content, indicating that chitosan oligosaccharide can promote protein deposition and synthesis in weaned piglets. Moreover, preliminary results from this study showed that compared with the control group, the growth rate of experimental groups 2 and 3 increased by 7.93% and 14.02%, respectively, and the feed-to-gain ratio decreased by 11.02% and 12.27%, respectively [25], further confirming these findings.

Serum ALP is closely related to the absorption and transport of fats, carbohydrates, and proteins [22,26]. Li et al. [27] found that chitosan oligosaccharide supplementation had no significant effect on ALP activity in swimming crabs. The present results showed that different chitosan oligosaccharide supplementation levels had no significant effect on serum ALP activity in piglets, consistent with the above study.

In conclusion: Chitosan oligosaccharide can significantly decrease serum AST and ALT activities and increase serum TP content in weaned piglets. Chitosan oligosaccharide can enhance humoral and cellular immunity in weaned piglets. Dietary supplementation with 50 and 150 g/t chitosan oligosaccharide can significantly increase serum IgA content, and significantly increase serum TNF-α content. Chitosan oligosaccharide can significantly improve serum antibody titers and health status in piglets. Dietary supplementation with 150 g/t chitosan oligosaccharide can significantly increase serum pseudorabies and PRRS antibody titers in weaned piglets.

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