Effects of Chitosan Oligosaccharide on Growth Performance, Serum Immune and Antioxidant Indices in Mice: Postprint

Authors: Zhang Nannan, Li Lanlan, Zhang Xiaoyun, Wen Zhengshun, Zou Xiaoting

Date: 2018-12-24T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of gavage administration of different doses of chitosan oligosaccharides (COS) on growth performance, serum immunoglobulin content, cytokine levels, and antioxidant enzyme activity in mice. Sixty 5-week-old specific pathogen-free (SPF) ICR male mice were selected and randomly divided into 6 groups, including 1 control group and 5 treatment groups, with 10 mice per group. Mice in the 5 treatment groups were administered COS (dissolved in physiological saline) via gavage at doses of 0.2, 0.4, 0.6, 0.8, and 1.0 g/kg BW, respectively, while the control group received an equal volume of physiological saline. The experimental period lasted 6 weeks. The results showed: 1) Gavage administration of different doses of COS had no significant effect on mouse body weight (P>0.05). 2) Administration of 0.6 g/kg BW COS significantly increased the thymus index in mice (P<0.05), which was 32.87% higher than that of the control group. 3) Administration of 0.6 g/kg BW COS significantly increased serum immunoglobulin A (IgA) and immunoglobulin M (IgM) contents in mice (P<0.05), which were increased by 12.14% and 20.69% compared with the control group, respectively. 4) Gavage administration of different doses of COS had no significant effect on serum interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-1 (IL-1), transforming growth factor- (TGF-), and tumor necrosis factor- (TNF-) levels in mice (P>0.05). 5) Administration of 0.6 g/kg BW COS significantly increased myeloperoxidase (MPO) activity in mouse serum, which was 27.61% higher than that of the control group (P<0.05), and administration of 0.8 g/kg BW COS significantly increased peroxidase (POD) activity in mouse serum, which was 45.08% higher than that of the control group (P<0.05). In conclusion, under the conditions of this experiment, COS could promote the development of immune organs and regulate serum immune and antioxidant indices in mice.

Full Text

Effects of Chitosan Oligosaccharide on Growth Performance, Serum Immune Indexes and Antioxidant Indexes of Mice

ZHANG Nannan[1], LI Lanlan[1], ZHANG Xiaoyun[1], WEN Zhengshun[2], ZOU Xiaoting[1]*

[1]Key Laboratory of Animal Nutrition and Feed Science of Agriculture, Feed Science Institute, Zhejiang University, Hangzhou 310058, China
[2]Zhejiang Ocean University, Zhoushan 316022, China

Abstract: This experiment was conducted to investigate the effects of gavage administration of different doses of chitosan oligosaccharide (COS) on growth performance, serum immunoglobulin contents, cytokine levels, and antioxidant enzyme activities in mice. Sixty 5-week-old specific pathogen-free (SPF) ICR male mice were selected and randomly divided into 6 groups, including 1 control group and 5 experimental groups with 10 mice each. The mice in the 5 experimental groups were administered COS (dissolved in normal saline) at doses of 0.2, 0.4, 0.6, 0.8, and 1.0 g/kg BW via gavage, while the control group received the same volume of normal saline. The experimental period lasted for 6 weeks. The results showed as follows: (1) Gavage administration of different doses of COS had no significant effect on mouse body weight (P>0.05). (2) Administration of 0.6 g/kg BW COS significantly increased the thymus index (P<0.05), which was 32.87% higher than that of the control group. (3) Administration of 0.6 g/kg BW COS significantly increased serum immunoglobulin A (IgA) and immunoglobulin M (IgM) contents (P<0.05), which were 12.14% and 20.69% higher than those of the control group, respectively. (4) No significant effects were observed on serum interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-1 (IL-1), transforming growth factor- (TGF-), and tumor necrosis factor- (TNF-) levels with different doses of COS (P>0.05). (5) Administration of 0.6 g/kg BW COS significantly increased serum myeloperoxidase (MPO) activity, which was 27.61% higher than that of the control group (P<0.05), while administration of 0.8 g/kg BW COS significantly increased serum peroxidase (POD) activity, which was 45.08% higher than that of the control group (P<0.05). In summary, under the conditions of this experiment, COS can promote the development of immune organs and regulate serum immune and antioxidant indexes in mice.

Keywords: chitosan oligosaccharides; ICR mice; growth performance; immunoglobulin; cytokine; antioxidant enzyme

Introduction

The prohibition of antibiotics and lack of effective vaccines have, to some extent, promoted the application of probiotics, prebiotics, immune enhancers, and plant extracts [1]. Chitosan oligosaccharide (COS), a type of prebiotic, is the only positively charged alkaline amino polysaccharide in nature [2]. It possesses good solubility and low viscosity at neutral pH, is not easily decomposed in the gastrointestinal tract, and can be directly absorbed into the bloodstream to exert its effects [3], attracting widespread attention from researchers. Recent studies have focused on reporting various biological effects of COS, including growth promotion, anti-infection, anti-tumor, blood pressure reduction, and cholesterol lowering. Research has shown that dietary supplementation with COS can increase intestinal villus height and reduce crypt depth in weaned piglets, thereby improving nutrient digestibility and positively affecting piglet growth performance [4]. Further studies have demonstrated that COS can promote the proliferation of *Bifidobacterium* and *Lactobacillus* in the intestines of juvenile *Jian* carp, improve intestinal structure, and consequently regulate growth and immune performance [5]. Numerous *in vitro* studies have shown that COS can stimulate macrophages to secrete cytokines such as tumor necrosis factor- (TNF-), interleukin-6 (IL-6), and interleukin-1 (IL-1) [6-8], thereby enhancing macrophage activity. However, few studies have reported whether COS can induce cytokine secretion in normal animals. Additionally, antioxidant research on COS in mammals has primarily focused on stress-prone periods such as late gestation sows and piglet stages [9], with limited studies conducted during normal periods. Therefore, this experiment was designed to investigate the effects of COS on growth performance, immune function, and antioxidant capacity in mice under normal physiological conditions, aiming to provide a theoretical basis for the production of functional foods using COS.

1.1 Experimental Materials

The COS used in this experiment was provided by Zhejiang Weifeng Biotechnology Co., Ltd., with a purity of 99%, molecular weight of 1,000 u, deacetylation degree of 85%-90%, polymerization degree of 5-10, water-soluble, yellow powdery solid, and production batch number KG-1503029.

1.2 Experimental Design and Management

The experimental animals were 4-week-old male SPF ICR mice weighing 14-16 g, purchased from Shanghai Laike Biotechnology Co., Ltd. (production license number: SCXK (Shanghai) 2002-0002). After one week of adaptation in randomly assigned cages, 60 healthy mice with uniform body weight were selected and randomly divided into 6 groups with 10 mice each. The experiment included 1 control group and 5 experimental groups with COS doses of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 g/kg BW, administered via gavage. During gavage, COS was dissolved in normal saline (NS) to prepare solutions of 0, 10, 20, 30, 40, and 50 mg/mL concentrations, and administered at 0.02 mL/g BW to the correspond-

ing dose groups daily at a fixed time. Body weight was measured weekly to control gavage volume according to weight. The gavage period lasted 42 days. The mice were housed in a semi-closed ventilation system environment at the Zhejiang University Laboratory Animal Center, with ambient temperature controlled at (24±1)°C and humidity at (50±10)%. They had free access to clean water and feed (basic mouse diet provided by the Zhejiang University Laboratory Animal Center), with bedding changed twice weekly. The mouse feeding experiment was conducted in accordance with the national "Regulations on the Administration of Laboratory Animals."

1.3.1 Body Weight Measurement

On days 1, 8, 15, 22, 29, 36, and 43 of the experiment, each mouse was weighed in the morning after fasting to analyze body weight changes.

1.3.2 Determination of Immune Organ Indexes

At the end of the experiment, after 24 h of fasting (with free access to water) and weighing, all 10 mice in each group were euthanized by cervical dislocation after blood collection via eyeball removal. The thymus and spleen were dissected, weighed, and used to calculate immune organ indexes.

Thymus index (mg/g) = thymus weight (mg) / body weight (g)
Spleen index (mg/g) = spleen weight (mg) / body weight (g)

1.3.3 Determination of Serum Immune Indexes

Blood collected from 10 mice in each group was allowed to stand at room temperature for 2 h, then centrifuged at 3,000 r/min for 10 min to collect serum for determination of immunoglobulin contents, cytokine levels, and antioxidant enzyme activities, with 10 replicates per group.

1.3.3.1 Determination of Serum Immunoglobulin Contents

Serum immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) contents were determined using assay kits purchased from Wuhan Boster Biological Engineering Co., Ltd., with reagent preparation and index determination performed according to the manufacturer' s instructions.

1.3.3.2 Determination of Serum Cytokine Levels

Serum IL-6, interleukin-10 (IL-10), IL-1 , transforming growth factor- (TGF-), and TNF- levels were determined using assay kits purchased from Wuhan Boster Biological Engineering Co., Ltd., with reagent preparation and index determination performed according to the manufacturer' s instructions.

1.3.3.3 Determination of Serum Antioxidant Enzyme Activities

Serum superoxide dismutase (SOD), myeloperoxidase (MPO), peroxidase (POD), and alkaline phosphatase (AKP) activities were determined using assay kits purchased from Nanjing Jiancheng Bioengineering Institute, with reagent

preparation and index determination performed according to the manufacturer' s instructions.

1.4 Data Analysis

Experimental data were analyzed using SPSS 20.0 statistical software. One-way ANOVA was used to compare statistical differences among groups. When significant differences were detected, Duncan' s multiple comparison test was applied. Results were expressed as mean ± standard error (SE), with $P<0.05$ indicating significant difference.

Results

2.1 Body Weight Changes in Each Group

During the 42-day COS gavage period, no abnormal reactions or deaths occurred in any group. At the end of the experiment, gavage administration of different COS doses had no significant effect on mouse growth performance ($P>0.05$) (Table 1). The body weight gains in the control group, 0.2 g/kg BW COS group, 0.4 g/kg BW COS group, 0.6 g/kg BW COS group, 0.8 g/kg BW COS group, and 1.0 g/kg BW COS group were 6.12, 5.51, 5.29, 5.15, 6.44, and 5.21 g, respectively. The 0.8 g/kg BW COS group showed a 5.23% higher weight gain than the control group, while other experimental groups showed slightly lower weight gains.

Table 1 Change of body weight of mice from different groups during the whole experimental period

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

2.2 Effects of COS on Immune Organ Indexes in Mice

As shown in Table 2 , the thymus index in the 0.6 g/kg BW COS group was significantly higher than that in the control group ($P<0.05$), with an increase of 32.87%. Other dose groups showed increased thymus indexes compared with the control group, but the differences were not significant ($P>0.05$). Gavage administration of different COS doses had no significant effect on spleen index ($P>0.05$).

Table 2 Effects of COS on immune organ indexes of mice

2.3 Effects of COS on Serum Immunoglobulin Contents in Mice

As shown in Table 3 , serum IgA, IgG, and IgM contents showed an overall trend of increasing first and then decreasing with increasing COS gavage dose, reaching maximum values at a dose of 0.6 g/kg BW. Specifically, serum IgA, IgG, and

IgM contents in the 0.6 g/kg BW group were 12.14% (P<0.05), 6.89% (P>0.05), and 20.69% (P<0.05) higher than those in the control group, respectively.

Table 3 Effects of COS on serum immune globulin contents of mice

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

2.4 Effects of COS on Serum Cytokine Levels in Mice

As shown in Table 4 , gavage administration of different COS doses had no significant effect on serum IL-6, IL-10, IL-1 , TGF- , and TNF- levels (P>0.05). However, serum IL-6, IL-1 , and TGF- levels in experimental groups showed an overall decreasing trend compared with the control group, reaching minimum values in the 0.6 g/kg BW group, which were 6.92%, 20.27%, and 9.34% lower than the control group, respectively (P>0.05). Serum IL-10 level showed an overall increasing trend compared with the control group, reaching a maximum value in the 0.4 g/kg BW group, which was 34.00% higher than the control group (P>0.05).

Table 4 Effects of COS on serum cytokine levels of mice

2.5 Effects of COS on Serum Antioxidant Enzyme Activities in Mice

As shown in Table 5 , COS gavage showed a trend of increasing serum SOD and POD activities. SOD activity was highest in the 0.4 g/kg BW group, showing an 11.31% increase compared with the control group (P>0.05). POD activity was highest in the 0.8 g/kg BW group, showing a 45.08% increase compared with the control group (P<0.05). Serum MPO and AKP activities showed a trend of increasing first and then decreasing with increasing COS dose. MPO activity was highest in the 0.6 g/kg BW COS group, showing a 27.61% increase compared with the control group (P<0.05). AKP activity was highest in the 0.4 g/kg BW group, showing a 21.30% increase compared with the control group (P>0.05).

Table 5 Effects of COS on serum antioxidant enzyme activities of mice

Discussion

3.1 Effects of COS on Mouse Growth Performance

Reports on the effects of COS on animal growth performance have been inconsistent. Some researchers believe that dietary COS supplementation can promote growth performance in piglets [10-11], while Qiao et al. [12] found that COS had no significant effect on growth performance of weaned piglets. These discrepancies may be attributed to differences in molecular weight and solubility of COS used in different experiments, as well as differences in animal species and living

environments. As discussed above, the growth-promoting effect of COS is unstable and greatly influenced by environmental sanitation conditions. Moreover, in studies where COS showed growth-promoting effects, growth performance did not increase linearly with COS dose but rather reached an optimum value before declining [13].

The results of this experiment showed that during the early stage (days 1-22), weight gain in the low-dose COS group (0.2 g/kg BW) was higher than that in the control group, while weight gain in the medium- and high-dose groups (0.6, 0.8, and 1.0 g/kg BW) was lower than that in the control group. This indicates that within the gavage dose range of 0.6-0.8 g/kg BW, COS showed a trend of improving growth performance in mice. However, when the COS dose reached 1.0 g/kg BW, growth performance showed a decreasing trend, consistent with the aforementioned research results. Evidently, there is an appropriate range for COS supplementation, and excessive supplementation may adversely affect animal growth performance. Currently, no studies have reported on the side effects of COS, and the specific reasons require further investigation. Current research suggests that mammalian digestion of carbohydrates is mainly limited to polysaccharides connected by -1,4 glycosidic bonds, with weak or no ability to decompose other forms of glycosidic bonds. Since COS is connected by -1,4 glycosidic bonds, it cannot be decomposed by digestive enzymes in the mammalian small intestine and is directly absorbed. Excessive supplementation may cause adverse diarrhea and negative effects on growth [14]. During the later stage of the experiment (days 22-43), weight gain in the medium- and high-dose groups (0.6 and 0.8 g/kg BW) was higher than that in the control group and low-dose groups (0.2 and 0.4 g/kg BW), possibly because mice developed adaptability to COS during the later stage. The specific reasons require further investigation.

3.2 Effects of COS on Mouse Immune Organ Indexes

Immune organs consist of central immune organs (bone marrow and thymus) and peripheral immune organs (lymph nodes, spleen, and mucosa-associated lymphoid tissue). The thymus is the primary site for T cell differentiation and maturation, inducing lymphocytes to produce mature immune cells for immune responses. The spleen is the largest peripheral immune organ and the main site for immune responses [15]. Thymus index and spleen index can serve as basic immune indicators that directly reflect immune function [16-17]. Current research suggests that COS has certain effects on immune organ development. The results of this experiment showed that gavage administration of 0.6 g/kg BW COS significantly increased the thymus index, and 0.6-0.8 g/kg BW COS showed a trend of increasing the spleen index. Deng et al. [18] reported that aloe polysaccharides could thicken the thymic cortex, clearly separate cortex and medulla, and make lymphocytes denser. It is speculated that the significant increase in thymus index in the 0.6 g/kg BW COS group in this experiment may be due to COS promoting lymphocyte development in the mouse thymus,

increasing the number of thymic lymphocytes, thereby promoting immune organ development and improving immune function.

3.3 Effects of COS on Serum Immunoglobulin Contents in Mice

In vitro studies have shown that COS can stimulate macrophages to secrete multifunctional cytokines, which stimulate the differentiation of T cells and B cells and promote immunoglobulin production [19]. The results of this experiment showed that gavage administration of 0.6 g/kg BW COS significantly increased serum IgA and IgM contents in mice. It is speculated that the active groups of COS may directly stimulate the mouse immune response [20]. Additionally, immunoglobulins are glycoproteins with chemical structures similar to or possessing antibody activity. Most sugars in their structure are bound in the form of glycans, which have two types: N-acetylgalactosamine and N-acetylglucosamine. COS can provide N-acetylglucosamine residues and bind to receptors of residues present on the cell surface with immune activity, thereby improving immune capacity. Furthermore, the positive charge carried by COS can attract the negative charges on the surface of T lymphocytes and macrophages, stimulating immune cells to send instructions to B cells to produce immunoglobulins [21]. Other studies have shown that beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* residing in the animal gastrointestinal tract can secrete enzymes that open -1,4 glycosidic bonds to utilize COS [4]. *Bifidobacterium* and *Lactobacillus* can utilize COS to produce succinic acid, lactic acid, and short-chain fatty acids, reducing intestinal pH and creating an acidic intestinal environment conducive to the proliferation of lactic acid bacteria [22]. Harmful bacteria in the intestine such as *Salmonella* and *Escherichia coli* cannot utilize COS because they cannot secrete enzymes to decompose it. COS can bind to anionic substances on bacterial cell walls or cell surfaces through its positively charged free amino groups, preventing nutrient entry or causing cell deformation, thereby interfering with normal metabolism and inhibiting harmful bacterial proliferation [23]. Therefore, COS may also improve immune function by promoting the growth of beneficial bacteria and inhibiting harmful bacteria.

3.4 Effects of COS on Serum Cytokine Contents in Mice

As an immune enhancer, COS can bind to specific receptors on lymphocytes or phagocytes and effectively stimulate the immune response through synergistic action with cytokines [24]. Cytokines released in this process include interleukins and interferons. Reports on the effects of COS on cytokine contents have been inconsistent. Deng et al. [25] showed that COS could stimulate macrophages to release IL-1 , IL-6, and TNF- , thereby improving immunity in broiler chickens. However, Walsh et al. [26] found that COS did not affect the expression of IL-6, IL-10, or TNF- in weaned piglets. The results of this experiment showed that serum IL-6, IL-1 , and TGF- contents in experimental groups showed an overall decreasing trend compared with the control group, while serum IL-10 content showed an overall increasing trend. The amino groups in the molecular

structure of COS can be recognized by the immune system, bind to receptors on macrophage surfaces, stimulate macrophages to release cytokines, and simultaneously induce the expression of interleukin-2 (IL-2) receptors on T cell surfaces, accelerating T cell maturation and IL-2 release. After binding to its receptor, IL-2 further accelerates T cell maturation and differentiation, stimulating antibody production in blood circulation [21]. Jia et al. [27] found that COS could significantly increase the proportion of CD8+ T lymphocytes in mouse spleens, and CD8+ T lymphocytes play an important role in IL-10 production [28]. IL-10 is a central regulator of the immune system *in vivo* [29]; its deficiency not only causes colonic inflammation in mice but also leads to epithelial permeability inflammation [30]. In this experiment, serum IL-10 content showed an overall increasing trend after gavage administration of different COS doses, which would not trigger inflammatory responses. As an important anti-inflammatory cytokine, IL-10 can inhibit the production of excessive inflammatory cytokines such as IL-6 and IL-1 by activated T cells, thereby preventing damage to the body from excessive immune responses [31]. This experiment showed that serum IL-6 and IL-1 contents in experimental groups showed an overall decreasing trend compared with the control group, which would not trigger excessive immune responses and was consistent with the increased serum IL-10 content in experimental groups. These results are consistent with the findings of Walsh et al. [26] but differ from those of Deng et al. [25]. Since pigs and mice are both mammals while chickens are birds, it is speculated that the effects of COS may differ between mammals and birds.

3.5 Effects of COS on Serum Antioxidant Enzyme Activities in Mice

In vitro determination of COS scavenging or inhibition rates against superoxide anion radicals (O ·), hydroxyl radicals (·OH), diphenylpicrylhydrazyl radicals (DPPH·), and other free radicals has demonstrated that COS has free radical scavenging and antioxidant functions. Xie et al. [32] used chemiluminescence technology to investigate the ·OH scavenging capacity of COS, finding it comparable to thiourea and higher than mannitol and benzoic acid. Wang et al. [33] found through *in vitro* antioxidant activity assays that when concentrations of ascorbic acid and COS reached 0.04 and 0.02 mg/mL, respectively, the DPPH· scavenging rate could reach 90%, indicating that COS has strong DPPH· scavenging capacity. With deepening research, antioxidant activity studies of COS at the cellular level have gradually been conducted. Zhang et al. [34] showed in N9 microglial cells that COS could inhibit lipopolysaccharide (LPS)-induced damage to N9 microglial cells by reducing reactive oxygen species (ROS) levels, thereby protecting nerve cells. Joodi et al. [35] demonstrated that COS could effectively reduce intracellular ROS levels and decrease oxidative damage to cells caused by hydrogen peroxide (H O). The antioxidant activity of COS has also been verified in animals, with more studies conducted in fish. Sun et al. [36] reported that dietary COS supplementation could significantly increase SOD and AKP activities in serum of juvenile GIFT tilapia. Li et al. [37] found that COS could significantly increase SOD and POD activities in serum of swim-

ming crabs. In mammals, no studies have reported on the effects of COS on antioxidant enzyme activities. SOD is one of the key antioxidant enzymes and a natural scavenger of superoxide radicals, capable of eliminating excess free radicals in the body and maintaining a dynamic balance between free radical formation and elimination, thereby preventing damage to biomolecules. SOD can serve as a non-specific immune indicator to evaluate the effect of immune stimulants on non-specific immunity. POD is widely present in animals, plants, and microorganisms and is one of the important enzymes in organisms. It can combine with H O to reduce free radical damage to the body and scavenge reactive oxygen species during cell metabolism, thereby improving disease resistance. AKP is universally present in animals and plants and is an important component of lysosomal enzymes, which play important roles in immune responses. MPO is an important oxidase that, together with H O and halides, forms an antimicrobial system that plays an important role in the immune system of higher animals [38]. Therefore, this experiment determined the effects of COS on SOD, MPO, POD, and AKP activities in mouse serum. The results showed that COS could significantly increase MPO and POD activities in mouse serum, with maximum values reached in the 0.6 g/kg BW and 0.8 g/kg BW groups, respectively. The amino groups, primary hydroxyl groups, and secondary hydroxyl groups in COS molecules are the structural basis for its antioxidant function [39]. Feng et al. [40] showed that the reducing end groups in COS structure could scavenge or inhibit free radicals through their reducing capacity. Xie et al. [32] demonstrated that amino groups in COS could combine with hydrogen ions in solution to form NH , and the hydrogen ions in NH could react with other free radicals to form stable substances, thereby scavenging free radicals. Therefore, it is speculated that COS can maintain relative stability of the internal environment by activating and improving the antioxidant defense system in the body.

Conclusion

Under the conditions of this experiment, gavage administration of different COS doses had no significant effects on mouse growth performance, spleen index, serum IgG content, cytokine levels, or SOD and AKP activities. However, at doses of 0.6-0.8 g/kg BW, COS showed a trend of increasing spleen index and significantly increased thymus index as well as serum IgA and IgM contents and MPO and POD activities to varying degrees.

References

[1] ELALA N M A, RAGAA N M. Eubiotic effect of a dietary acidifier (potassium diformate) on health status cultured *Oreochromis niloticus* [J]. Journal of Advanced Research, 2015, 6(4): 621-629.
[2] WAN J, JIANG F, XU Q S, et al. New insights into the role of chitosan oligosaccharide in enhancing growth performance, antioxidant capacity, immunity and intestinal development of weaned pigs [J]. RSC Advances, 2017, 7(16):

9669-9679.
[3] 谭利伟, 管昶, 周鲁宁, 等. 壳寡糖在动物营养中的研究与应用 [J]. 中国畜牧杂志, 2016, 52(17): 91-97.
[4] 黄鑫玮, 杨莎莎, 刘毅, 等. 壳寡糖对幼建鲤生长性能、脂肪代谢、非特异性免疫功能和肠道健康的影响 [J]. 动物营养学报, 2015, 27(7): 2106-2114.
[5] ZHOU T X, CHO J H, KIM I H. Effects of supplementation of chito-oligosaccharide on the growth performance, nutrient digestibility, blood characteristics and appearance of diarrhea in weanling pigs [J]. Livestock Science, 2012, 144(3): 263-268.
[6] YOON H J, MOON M E, IM S Y, et al. Chitosan oligosaccharide (COS) inhibits LPS-induced inflammatory effects in RAW 264.7 macrophage cells [J]. Biochemical & Biophysical Research Communications, 2007, 358(3): 954-959.
[7] MA P, LIU H T, WEI P, et al. Chitosan oligosaccharides inhibit LPS-induced over-expression of IL-6 and TNF- in RAW264.7 macrophage cells through blockade of mitogen-activated protein kinase (MAPK) PI3K/Akt signaling pathways [J]. Carbohydrate Polymers, 2011, 84(4): 1391-1398.
[8] 麻攀, 于炜婷, 魏鹏, 等. 壳寡糖作用于巨噬细胞的机制初探 [J]. 天然产物研究与开发, 2012(5): 660-662.
[9] 龙次民, 谢春艳, 吴信, 等. 妊娠后期母猪饲粮中添加壳寡糖对新生仔猪抗氧化能力的影响 [J]. 动物营养学报, 2015, 27(4): 1207-1213.
[10] 张玲, 陈代文, 杨继文, 等. 壳寡糖对环磷酰胺应激仔猪生产性能和免疫功能的影响 [J]. 中国畜牧杂志, 2013, 49(17): 58-62.
[11] 李阳, 常文环, 张姝, 等. 饲粮添加壳寡糖和干酪乳杆菌对肉鸡生长性能、肌肉品质及抗氧化性能的影响 [J]. 动物营养学报, 2016, 28(5): 1450-1461.
[12] 乔丽红, 赵颖, 倪红玉, 等. 低聚壳聚糖对断奶仔猪血清生化指标、抗氧化性能和粪便微生物的影响 [J]. 粮食与饲料工业, 2013(3): 47-50.
[13] 徐小龙, 周鲁宁, 张斌, 等. 壳寡糖替代抗生素对肉仔鸡生长性能及免疫功能的影响 [J]. 饲料广角, 2016(17): 33-37.
[14] 黄钦成, 申光荣, 谭北平, 等. 饲料中添加壳寡糖和/或霉菌毒素吸附剂对凡纳滨对虾生长性能、非特异性免疫力及抗病力的影响 [J]. 动物营养学报, 2017, 29(11): 4036-4047.
[15] 侯改凤, 李瑞, 黄兴国. 寡糖的免疫调节作用及其在动物生产中的应用 [J]. 中国饲料, 2015(4): 28-30.
[16] CHEN B, LIU L F, XU H Y, et al. Effectiveness of immune therapy combined with chemotherapy on the immune function and recurrence rate of cervical cancer [J]. Experimental and Therapeutic Medicine, 2015, 9(3): 1063-1067.
[17] YU G P, CHEN G Q, HUANG B, et al. Effect of early enteral nutrition on postoperative nutritional status and immune function in elderly patients with esophageal cancer or cardiac cancer [J]. Chinese Journal of Cancer Research, 2013, 25(3): 299-305.
[18] 邓阳勇, 伍参荣, 杨旭丽, 等. 芦荟多糖对衰老小鼠胸腺组织结构的影响 [J]. 中南医学科学杂志, 2011, 39(6): 626-628.
[19] DANG Y B, LI S, WANG W X, et al. The effects of chitosan oligosaccharide on the activation of murine spleen CD11c+ dendritic cells via Toll-like receptor 4 [J]. Carbohydrate Polymers, 2011, 83(3): 1075-1081.
[20] 崔健, 康鹏天. 壳寡糖的生理功能及在水产养殖中的作用 [J]. 甘肃畜牧兽医, 2015, 45(4): 21-24.

[21] 陈虹, 臧素敏, 侯伟革. 不同浓度壳寡糖对蛋用鹌鹑免疫功能的影响 [J]. 中国畜牧兽医, 2013, 40(1): 108-111.
[22] 占秀安, 胡彩虹, 许梓荣. 果寡糖对肉鸡生长、肠道菌群和肠形态的影响 [J]. 中国兽医学报, 2003, 23(2): 196-198.
[23] 孙忠保, 阎宏, 马永军. 寡糖在动物生产中的应用 [J]. 农业科学研究, 2004, 25(4): 80-84.
[24] 孟晓, 王纪亭, 万文菊, 等. 低分子质量壳寡糖对蛋鸡生产性能、蛋品质、血清生化指标、盲肠微生物数量及脾脏白细胞介素-2、肿瘤坏死因子- 基因表达的影响 [J]. 动物营养学报, 2017, 29(5): 1590-1599.
[25] DENG X Z, LI X J, LIU P, et al. Effect of chito-oligosaccharide supplementation on immunity broiler chickens [J]. Asian-Australasian Journal Animal Sciences, 2008, 21(11): 1651-1658.
[26] WALSH A M, SWEENEY T, BAHAR B, et al. The effect of chitooligosaccharide supplementation on intestinal morphology, selected microbial populations, volatile fatty acid concentrations immune expression weaned pig [J]. Animal, 2012, 6(10): 1620-1626.
[27] 贾培媛, 李海霞, 巫亚俊, 等. 壳寡糖对猪蓝耳病灭活疫苗的佐剂活性研究 [C]//中国酶工程与糖生物工程学术研讨会论文摘要集. 镇江: 中国微生物学会酶工程专业委员会, 2015: 66-68.
[28] GELFANOVA V, LAI Y, GELFANOV V, et al. Modulation of cytokine responses of murine CD8+ intestinal intraepithelial lymphocytes by IL-4 and IL-12 [J]. Journal of Biomedical Science, 1999, 6(4): 269-276.
[29] BERG D J, ZHANG J, WEINSTOCK J V, et al. Rapid development of colitis in NSAID-treated IL-10-deficient mice [J]. Gastroenterology, 2002, 123(5): 1527-1542.
[30] BERG D J, DAVIDSON N, KÜHN R, et al. Enterocolitis and colon cancer interleukin-10-deficient mice are associated with aberrant cytokine production and CD4+ TH1-like responses [J]. Journal of Clinical Investigation, 1996, 98(4): 1010-1020.
[31] SUN X Y, YANG H, NOSE K, et al. Decline in intestinal mucosal IL-10 expression and decreased intestinal barrier function a mouse model total parenteral nutrition [J]. American Journal Physiology: Gastrointestinal Liver Physiology, 2008, 294(1): G139-G147.
[32] XIE W M, XU P X, LIU Q. Antioxidant activity of water-soluble chitosan derivatives [J]. Bioorganic & Medicinal Chemistry Letters, 2001, 11(13): 1699-1701.
[33] 王振伟, 申森. 超声波辅助酶法制备壳寡糖及抗氧化活性研究 [J]. 中国食品添加剂, 2014(4): 70-75.
[34] 张吉, 刘洪涛, 李秀英, 等. 壳寡糖对自由基的清除及对 N9 小胶质细胞的保护作用 [J]. 食品科学, 2010, 31(7): 81-85.
[35] JOODI G, ANSARI N, KHODAGHOLI F. Chitooligosaccharide-mediated neuroprotection is associated with modulation of Hsps expression reduction of MAPK phosphorylation [J]. International Journal of Biological Macromolecules, 2011, 48(5): 726-735.
[36] 孙立威, 文华, 蒋明, 等. 壳寡糖对吉富罗非鱼幼鱼生长性能、非特异性免疫及血液学指标的影响 [J]. 广东海洋大学学报, 2011, 31(3): 43-49.
[37] 李振达, 陈小娥, 廖智, 等. 壳寡糖对三疣梭子蟹免疫力的影响 [J]. 浙江海洋学院学报（自然科学版）, 2011, 30(1): 27-32.

[38] 李桂峰, 钱沛锋, 孙际佳, 等. 维生素 C 对胡子鲶血清免疫相关酶活性的影响 [J]. 大连水产学院报, 2004, 19(4): 301-305.
[39] 钟佳, 刘进辉, 肖定福, 等. 壳寡糖及其衍生物的抗氧化活性 [J]. 动物医学进展, 2015, 36(7): 118-121.
[40] FENG T, DU Y M, LI J, et al. Antioxidant activity of half N-acetylated water-soluble chitosan *in vitro* [J]. European Food Research and Technology, 2007, 225(1): 133-138.

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