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

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Abstract

This study aimed to investigate the effects of intragastric administration of different doses of chitosan oligosaccharides (COS) on growth performance, serum immunoglobulin content, 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 per group. The mice in the 5 experimental groups were administered COS (dissolved in physiological saline) by intragastric 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 for 6 weeks. The results showed: 1) Intragastric administration of different doses of COS had no significant effect on mouse body weight ($P>0.05$). 2) Intragastric 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) Intragastric 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 12.14% and 20.69% higher than those of the control group, respectively. 4) Intragastric 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) Intragastric 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 ($P<0.05$); intragastric 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 in mice and regulate serum immune and antioxidant indices.

Full Text

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

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Abstract: This experiment investigated the effects of gavage administration of different doses of chitosan oligosaccharide (COS) on growth performance, serum immunoglobulin content, cytokine levels, and antioxidant enzyme activities in mice. Sixty 5-week-old specific pathogen-free (SPF) ICR male mice were randomly divided into six groups, including one control group and five experimental groups, with ten mice per group. The five experimental groups received COS (dissolved in normal saline) at doses of 0.2, 0.4, 0.6, 0.8, and 1.0 g/kg body weight (BW) via gavage, while the control group received an equivalent volume of normal saline. The experimental period lasted six weeks. The results showed that: (1) gavage administration of different COS doses 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) different COS doses had no significant effects on serum interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-1 (IL-1), transforming growth factor- (TGF-), or tumor necrosis factor- (TNF-) levels ($P>0.05$); and (5) administration of 0.6 g/kg BW COS significantly increased serum myeloperoxidase (MPO) activity by 27.61% compared with the control group ($P<0.05$), while administration of 0.8 g/kg BW COS significantly increased serum peroxidase (POD) activity by 45.08% compared with the control group ($P<0.05$). In summary, under the conditions of this experiment, COS promoted immune organ development and regulated serum immune and antioxidant indexes in mice.

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

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Introduction

The prohibition of antibiotics and lack of effective vaccines have promoted the application of probiotics, prebiotics, immunoenhancers, and plant extracts to

some extent. Chitosan oligosaccharide (COS), a type of prebiotic, is the only naturally occurring alkaline amino polysaccharide with a positive charge. It possesses good solubility and low viscosity at neutral pH, is not easily degraded in the gastrointestinal tract, and can be directly absorbed into the bloodstream to exert its effects, attracting widespread research attention. Recent studies have focused on various biological effects of COS, including growth promotion, anti-infection, anti-tumor, blood pressure reduction, and cholesterol lowering. Dietary COS supplementation has been shown to increase intestinal villus height and reduce crypt depth in weaned piglets, thereby improving nutrient digestibility and positively affecting piglet growth performance. Further research has demonstrated that COS can promote the proliferation of *Bifidobacterium* and *Lactobacillus* in the intestines of juvenile Jian carp, improve intestinal structure, and regulate growth and immune performance. 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), thereby enhancing macrophage activity, though few studies have reported whether COS can induce cytokine secretion in normal animals. Additionally, research on the antioxidant effects of COS in mammals has primarily focused on stress-prone periods such as late gestation in sows and piglet stages, with limited studies conducted during normal periods. Therefore, this experiment aimed to investigate the effects of COS on growth performance, immune function, and antioxidant capacity in mice under normal physiological conditions, providing a theoretical basis for the production of functional foods using COS.

Materials and Methods

1.1 Experimental Material 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%, and degree of polymerization of 5–10. It was a yellow powder soluble in water, with 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(Hu)2002-0002). Sixty-five mice were randomly housed for a one-week adaptation period, after which sixty healthy mice with uniform body weight were selected and randomly divided into six groups of ten mice each. The experiment included one control group and five experimental groups receiving COS doses of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 g/kg BW via 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 daily at a fixed time. Body weight was measured weekly to control gavage volume. The gavage period lasted 42 days. Mice were housed in a semi-closed ventilation system at the Zhejiang University Laboratory Animal Center, with environmental temperature controlled at (24±1)°C and humidity at (50±10)%. Mice had free access to clean water and feed (basal diet

provided by the Zhejiang University Laboratory Animal Center). Bedding was changed twice weekly. All procedures were conducted in accordance with the national "Regulations on the Administration of Laboratory Animals."

1.3 Measurement Indicators and Methods

1.3.1 Body Weight Measurement Each mouse was weighed on an empty stomach on days 1, 8, 15, 22, 29, 36, and 43 of the experiment 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 ten mice in each group were euthanized by cervical dislocation following blood collection via eyeball removal. The thymus and spleen were dissected and weighed 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 ten mice per 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 content, cytokine levels, and antioxidant enzyme activities, with ten replicates per group.

1.3.3.1 Serum Immunoglobulin Content Determination
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., following the manufacturer' s instructions for reagent preparation and measurement.

1.3.3.2 Serum Cytokine Level Determination
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., following the manufacturer' s instructions for reagent preparation and measurement.

1.3.3.3 Serum Antioxidant Enzyme Activity Determination
Serum superoxide dismutase (SOD), myeloperoxidase (MPO), peroxidase (POD), and alkaline phosphatase (AKP) activities were determined using assay kits purchased from Nanjing Jiancheng Bioengineering Institute, following the manufacturer' s instructions for reagent preparation and measurement.

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. Duncan' s multiple comparison test was applied when significant

differences were detected. Results are expressed as mean ± standard error (SE), with P<0.05 indicating significant difference.

Results

2.1 Body Weight Changes of Mice in Each Group During the 42-day 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). Body weight gains in the control, 0.2, 0.4, 0.6, 0.8, and 1.0 g/kg BW COS groups 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% increase in body weight gain compared with the control group, while other experimental groups showed slight decreases.

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

COS gavage dose (g/kg BW)	Day 1	Day 8	Day 15	Day 22	Day 29	Day 36	Day 43
0 (Control)	29.90±0.51	31.96±0.36	33.08±0.80	34.72±0.77	36.94±0.82	38.72±0.76	40.02±0.76
0.2	29.27±0.51	31.71±0.36	32.93±0.70	34.23±0.73	35.80±0.74	37.51±0.70	38.78±0.80
0.4	29.51±0.61	31.71±0.38	32.62±1.03	33.97±0.73	35.63±0.40	36.77±0.58	38.80±0.53
0.6	29.66±0.33	31.92±1.32	33.20±1.63	35.10±1.33	36.15±0.84	37.27±0.57	37.81±0.56
0.8	29.21±1.34	31.49±0.32	32.30±0.89	34.13±0.69	35.83±0.82	37.11±0.85	38.65±0.81
1.0	29.11±0.51	31.31±0.42	32.11±0.93	34.17±0.74	35.87±0.68	38.17±1.00	40.32±0.90

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 of Mice As shown in Table 2 , the 0.6 g/kg BW COS group showed a significant increase in thymus index compared with the control group (P<0.05), with a 32.87% improvement. 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

Items	COS gavage dose (g/kg BW)	Thymus index (mg/g)	Spleen index (mg/g)
	0 (Control)	1.46±0.14b	2.62±0.05
	0.2	1.66±0.07ab	2.74±0.16
	0.4	1.76±0.07ab	2.76±0.08
	0.6	1.94±0.14a	2.99±0.06

Items	COS gavage dose (g/kg BW)	Thymus index (mg/g)	Spleen index (mg/g)
	0.8	1.92±0.11ab	2.80±0.09
	1.0	1.51±0.14ab	2.80±0.19

2.3 Effects of COS on Serum Immunoglobulin Contents of Mice As shown in Table 3 , serum IgA, IgG, and IgM contents generally increased initially and then decreased with increasing COS dose, reaching maximum values at 0.6 g/kg BW. Specifically, the 0.6 g/kg BW group showed increases of 12.14% (P<0.05), 6.89% (P>0.05), and 20.69% (P<0.05) in serum IgA, IgG, and IgM contents, respectively, compared with the control group.

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

COS gavage dose (g/kg BW)	IgA (ng/mL)	IgG (g/mL)	IgM (g/mL)
0 (Control)	388.77±22.48b	217.86±19.31	1.16±0.34b
0.2	404.65±14.92ab	225.61±51.21	1.24±0.16ab
0.4	418.76±10.90ab	217.71±18.76	1.34±0.31ab
0.6	435.98±10.03a	232.88±18.43	1.40±0.25a
0.8	416.60±11.78ab	221.19±16.34	1.28±0.11ab
1.0	404.82±8.96ab	226.16±13.00	1.22±0.08ab

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 of 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- , or TNF- levels (P>0.05). However, serum IL-6, IL-1 , and TGF- levels in experimental groups generally showed a decreasing trend compared with the control group, reaching minimum values in the 0.6 g/kg BW group, with reductions of 6.92%, 20.27%, and 9.34%, 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, with a 34.00% increase (P>0.05).

Table 4 Effects of COS on serum cytokine levels of mice

COS gavage dose (g/kg BW)	IL-6 (ng/L)	IL-10 (pg/L)	IL-1 (ng/L)	TGF- (ng/L)	TNF- (ng/L)
0 (Control)	249.16±12.11	400±0.11	119.88±12.72	267.40±20.50	419.20±18.79
0.2	224.01±20.01	113±0.10	97.99±9.61	152.08±29.24	424.61±7.38
0.4	239.49±31.81	134±0.09	107.64±11.75	160.27±9.21	451.99±18.15

COS gavage dose (g/kg BW)	IL-6 (ng/L)	IL-10 (pg/L)	IL-1 (ng/L)	TGF- (ng/L)	TNF- (ng/L)
0.6	231.93±31.35	29±0.20	95.58±4.59	151.76±14.27	439.23±20.46
0.8	243.12±38.26	33±0.17	105.37±10.20	155.71±6.98	414.53±26.38
1.0	243.42±42.39	95±0.14	96.47±14.99	157.93±10.24	416.20±17.17

2.5 Effects of COS on Serum Antioxidant Enzyme Activities of Mice As shown in Table 5 , COS administration tended to increase serum SOD and POD activities in mice. SOD activity reached its highest level in the 0.4 g/kg BW group, with an 11.31% increase compared with the control group (P>0.05). POD activity reached its highest level in the 0.8 g/kg BW group, with a 45.08% increase compared with the control group (P<0.05). Serum MPO and AKP activities showed an initial increase followed by a decrease with increasing COS dose. MPO activity reached its maximum in the 0.6 g/kg BW COS group, with a 27.61% increase compared with the control group (P<0.05). AKP activity reached its maximum in the 0.4 g/kg BW group, with a 21.30% increase compared with the control group (P>0.05).

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

COS gavage dose (g/kg BW)	SOD (U/mg prot)	MPO (U/L)	POD (U/mL)	AKP (King' s unit/dL)
0 (Control)	101.21±11.09	9.67±0.90b	13.02±0.71b	2.77±0.11
0.2	106.88±7.86	9.97±0.52ab	15.64±1.27ab	2.75±0.13
0.4	112.65±4.01	11.17±0.68a	16.84±1.11ab	3.36±0.20
0.6	106.32±10.25	12.34±0.19a	15.04±1.20ab	3.29±0.17
0.8	109.91±9.58	9.47±0.78b	18.89±2.08a	3.25±0.23
1.0	108.39±7.22	9.36±0.74b	16.88±1.96ab	2.72±0.14

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 promotes piglet growth performance, while others found no significant effect on weaned piglets. These discrepancies may be attributed to differences in COS molecular weight and solubility among studies, as well as variations in animal species and living environments. The above discussion suggests that the growth-promoting effect of COS is unstable and greatly influenced by environmental sanitation conditions. Moreover, studies demonstrating growth-promoting effects of COS have shown that performance does not increase linearly with dose but rather reaches an optimum value before declining.

In this experiment, during the early stage (days 1–22), low-dose COS groups (0.2 g/kg BW) showed higher body weight gain than the control group, while

medium- and high-dose groups (0.6, 0.8, and 1.0 g/kg BW) showed lower weight gain. This indicates that within the gavage dose range of 0.6–0.8 g/kg BW, COS tended to improve mouse growth performance, but when the dose reached 1.0 g/kg BW, growth performance tended to decrease instead. These findings are similar to those reported previously. Evidently, COS supplementation has an appropriate dosage range, and excessive supplementation may adversely affect animal growth performance. Currently, no studies have reported side effects of COS, and the specific mechanisms 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 degradation of other glycosidic bonds. COS is connected by -1,4-glycosidic bonds and cannot be degraded by digestive enzymes in the mammalian small intestine, being directly absorbed instead. Excessive supplementation may cause adverse diarrhea and negative growth effects. During the later experimental stage (days 22–43), medium- and high-dose groups (0.6 and 0.8 g/kg BW) showed higher body weight gain than the control and low-dose groups (0.2 and 0.4 g/kg BW), possibly because mice developed adaptation to COS during the later stage, though the specific mechanisms require further study.

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, while the spleen is the largest peripheral immune organ and the main site for immune responses. Thymus and spleen indexes serve as basic immune indicators that directly reflect immune function. Current evidence suggests that COS affects immune organ development. This experiment showed that 0.6 g/kg BW COS significantly increased thymus index, and 0.6–0.8 g/kg BW COS tended to increase spleen index. Previous research has shown that aloe polysaccharides can thicken thymic cortex, clearly separate cortex and medulla, and increase lymphocyte density. We hypothesize that the significant increase in thymus index in the 0.6 g/kg BW COS group may be due to COS promoting lymphocyte development in the mouse thymus, increasing thymic lymphocyte numbers, thereby promoting immune organ development and enhancing immune function.

3.3 Effects of COS on Mouse Serum Immunoglobulin Contents *In vitro* studies have shown that COS can stimulate macrophages to secrete multifunctional cytokines that promote T cell and B cell differentiation and immunoglobulin production. This experiment demonstrated that 0.6 g/kg BW COS significantly increased serum IgA and IgM contents in mice. We speculate that the active groups of COS may directly stimulate the mouse immune response. Additionally, immunoglobulins are glycoproteins with chemical structures similar to or possessing antibody activity, with most sugars bound in the form of glycans. There are two types of glycans: N-acetylgalactosamine and

N-acetylglucosamine. COS can provide N-acetylglucosamine residues and bind to active residue receptors on cell surfaces, thereby enhancing immune capacity. Furthermore, the positive charge carried by COS can attract the negative charges on T lymphocyte and macrophage surfaces, activating immune cells to instruct B cells to produce immunoglobulins. Studies have also 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. The utilization of COS by *Bifidobacterium* and *Lactobacillus* can produce succinic acid, lactic acid, and short-chain fatty acids, reducing intestinal pH and creating an acidic intestinal environment favorable for *Lactobacillus* proliferation. Harmful intestinal bacteria such as *Salmonella* and *Escherichia coli* cannot utilize COS because they cannot secrete enzymes to degrade 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 bacterial metabolism and inhibiting harmful bacterial proliferation. Therefore, COS may also enhance immune function by promoting beneficial bacteria growth and inhibiting harmful bacteria.

3.4 Effects of COS on Mouse Serum Cytokine Levels As an immune enhancer, COS can bind to specific receptors on lymphocytes or phagocytes and effectively stimulate immune responses through synergistic action with cytokines. This process releases cytokines including interleukins and interferons. Reports on the effects of COS on cytokine levels have been inconsistent. Some studies have shown that COS can stimulate macrophages to release IL-1 , IL-6, and TNF- , thereby improving broiler chicken immunity, while other studies found that COS did not affect IL-6, IL-10, or TNF- expression in weaned piglets. This experiment showed that serum IL-6, IL-1 , and TGF- levels in experimental groups generally decreased compared with the control group, while serum IL-10 level showed an overall increasing trend. The amino groups in COS molecular structure can be recognized by the immune system and bind to macrophage surface receptors, stimulating macrophages to release cytokines while inducing interleukin-2 (IL-2) receptor expression on T cell surfaces, accelerating T cell maturation and IL-2 release. The binding of IL-2 to its receptors further accelerates T cell maturation and differentiation, stimulating antibody production in blood circulation. Previous research has found that COS can significantly increase the proportion of CD8+ T lymphocytes in mouse spleen, and CD8+ T lymphocytes play an important role in IL-10 production. IL-10 is a central regulator of the immune system, and its deficiency can cause not only colonic inflammation but also epithelial permeability inflammation in mice. In this experiment, serum IL-10 level 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 activated T cells from producing excessive inflammatory cytokines such as IL-6 and IL-1 , thereby preventing damage from excessive immune responses. This exper-

iment showed that serum IL-6 and IL-1 levels in experimental groups generally decreased compared with the control group, which would not trigger excessive immune responses and was consistent with the increased serum IL-10 levels in experimental groups. These results align with some previous studies in pigs but differ from those in chickens, suggesting that COS effects may vary between mammals and birds.

3.5 Effects of COS on Antioxidant Enzyme Activities in Mouse Serum

In vitro determination of COS scavenging rates or inhibition rates against superoxide anion radicals (O ·), hydroxyl radicals (·OH), diphenylpicrylhydrazyl radicals (DPPH·), and other free radicals has demonstrated COS' s free radical scavenging and antioxidant functions. Studies using chemiluminescence technology have shown that COS' s ·OH scavenging capacity is comparable to thiourea and higher than mannitol and benzoic acid. Other research has found that when ascorbic acid and COS concentrations reach 0.04 and 0.02 mg/mL, respectively, the DPPH· scavenging rate can reach 90%, indicating strong DPPH· scavenging ability. With deepening research, antioxidant activity studies of COS at the cellular level have gradually emerged. Studies on N9 microglial cells have shown that COS can protect nerve cells by reducing reactive oxygen species (ROS) levels and inhibiting lipopolysaccharide (LPS)-induced N9 microglial cell damage. Other research has demonstrated that COS can effectively reduce intracellular ROS levels and decrease oxidative damage caused by hydrogen peroxide (H O). The antioxidant activity of COS has also been verified in animals, with more studies conducted in fish. Dietary COS supplementation has been reported to significantly increase serum SOD and AKP activities in juvenile GIFT tilapia, and other studies have found that COS can significantly increase serum SOD and POD activities in swimming crabs. However, no studies have reported the effects of COS on antioxidant enzyme activities in mammals.

SOD is a key antioxidant enzyme and a natural scavenger of superoxide radicals that can eliminate excess free radicals and maintain 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 effects of immune stimulants on non-specific immunity. POD is widely present in animals, plants, and microorganisms and is an important enzyme that can combine with H O to reduce free radical damage, scavenge reactive oxygen species during cell metabolism, and enhance disease resistance. AKP is universally present in animals and plants and is an important component of lysosomal enzymes, which play crucial roles in immune responses. MPO is an important oxidase that, together with H O and halides, forms an antimicrobial system that plays important roles in the immune systems of higher animals. Therefore, this experiment determined the effects of COS on serum SOD, MPO, POD, and AKP activities in mice. The results showed that COS significantly increased serum MPO and POD activities, reaching maximum values in the 0.6 and 0.8 g/kg BW groups, respectively. The amino, primary hydroxyl, and secondary hydroxyl groups in COS molecules constitute the structural basis for

its antioxidant function. Studies have shown that the reducing end groups in COS structure can scavenge or inhibit free radicals through their reducing properties, and COS amino groups can combine with hydrogen ions in solution to form NH , which can react with other free radicals to form stable substances, thereby scavenging free radicals. Therefore, we hypothesize that COS can maintain relative stability of the internal environment by activating and enhancing the antioxidant defense system.

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 tended to increase spleen index and significantly increased thymus index as well as serum IgA and IgM contents and MPO and POD activities to varying degrees.

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