Effects of Chitosan Oligosaccharide on Growth Performance, Intestinal Histology, and Non-Specific Immune Function in Gibel Carp (Postprint)

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Date: 2018-12-25T00:00:00+00:00

Abstract

This experiment was conducted to investigate the effects of chitosan oligosaccharides (COS) on growth performance, intestinal histomorphology, and non-specific immune function in gibel carp (Carassius auratus gibelio). Eight experimental diets were formulated by supplementing 0, 0.02%, 0.04%, and 0.06% COS to basal diets containing either normal soybean oil or oxidized soybean oil, designated as CG, OO, CG-200, CG-400, CG-600, OO-200, OO-400, and OO-600, respectively. The diet containing normal soybean oil without COS supplementation (CG) served as the positive control group, while the diet containing oxidized soybean oil without COS supplementation (OO) served as the negative control group. A total of 960 gibel carp with an initial body weight of (7.60±0.05) g were randomly allocated into 8 groups, with 3 replicates (cages) per group and 40 fish per replicate, and cultured in pond cages for 72 days. The results demonstrated that: compared with the CG group, the specific growth rate (SGR) of the CG-200 group increased by 16.20% ($P<0.05$), whereas no significant differences were observed in the CG-400 and CG-600 groups ($P>0.05$); the CG-200 group exhibited increased intestinal fold height by 86.84% ($P<0.05$), increased intestinal wall thickness by 20.45% ($P<0.05$), decreased intestinal fold width by 12.18% ($P>0.05$), increased serum superoxide dismutase (SOD) activity by 6.59% ($P>0.05$), and decreased serum malondialdehyde (MDA) content by 16.94% ($P>0.05$); the OO group showed decreased SGR by 8.10% ($P<0.05$), decreased intestinal fold height by 17.81% ($P<0.05$), increased intestinal fold width by 70.37% ($P<0.05$), decreased intestinal wall thickness by 30.33% ($P>0.05$), decreased serum SOD activity by 27.35% ($P<0.05$), and increased serum MDA content by 25.72% ($P<0.05$). Compared with the OO group, the OO-200, OO-400, and OO-600 groups showed no significant increase in SGR

(P>0.05), among which the OO-400 group exhibited increased intestinal fold height by 18.89% (P<0.05), decreased intestinal fold width by 42.99% (P<0.05), increased intestinal wall thickness by 38.35% (P>0.05), increased serum SOD activity by 40.90% (P<0.05), and decreased serum MDA content by 16.20% (P>0.05). The OO-400 group achieved the same level as the CG group in terms of SGR, intestinal structure, serum SOD activity, and MDA content. Based on the results obtained under the experimental conditions, it was concluded that: supplementation of 0.02% COS in a conventional diet containing 4% soybean oil could promote growth, improve intestinal structure, and enhance non-specific immunity in gibel carp; oxidized soybean oil exerted negative effects on growth and health of gibel carp, and supplementation of 0.04% COS in this diet could ameliorate the negative effects caused by oxidized soybean oil, restoring the health status of gibel carp to normal levels.

Full Text

Effects of Chitosan Oligosaccharide on Growth Performance, Intestinal Structure and Non-Specific Immune Function of Crucian Carp (Carassius auratus gibelio)

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Abstract: This study investigated the effects of chitosan oligosaccharides (COS) on growth performance, intestinal structure, and non-specific immune function of crucian carp (Carassius auratus gibelio). Eight experimental diets were formulated by supplementing 0%, 0.02%, 0.04%, and 0.06% COS into basal diets containing either normal soybean oil or oxidized soybean oil, designated as CG, OO, CG-200, CG-400, CG-600, OO-200, OO-400, and OO-600, respectively. The diet without COS containing normal soybean oil (CG) served as the positive control, while the diet without COS containing oxidized soybean oil (OO) served as the negative control. A total of 960 crucian carp with initial body weight of (7.60±0.05) g were randomly divided into 8 groups with 3 replicates (net cages) per group and 40 fish per replicate, and cultured in pond net cages for 72 days. The results showed that compared with the CG group, the specific growth rate (SGR) of the CG-200 group increased by 16.20% (P<0.05), while no significant changes were observed in the CG-400 and CG-600 groups (P>0.05). The CG-200 group exhibited increased intestinal fold height by 86.84% (P<0.05) and intestinal wall thickness by 20.45% (P<0.05), decreased intestinal fold width by 12.18% (P>0.05), increased serum superoxide dismutase (SOD) activity by 6.59% (P>0.05), and decreased serum malondialdehyde (MDA) content by 16.94% (P>0.05). The OO group showed decreased SGR by 8.10% (P<0.05), reduced intestinal fold height by 17.81% (P<0.05), increased intestinal fold width by 70.37% (P<0.05), decreased intestinal wall thickness by 30.33% (P>0.05), reduced serum SOD activity by 27.35% (P<0.05), and increased serum MDA content by 25.72% (P<0.05). Compared with the OO group, the OO-200, OO-400, and OO-600 groups showed no significant increase in SGR (P>0.05). Specifically, the OO-400 group exhibited increased intestinal fold height by 18.89% (P<0.05), decreased intestinal fold width by 42.99% (P<0.05), increased intestinal wall thickness by 38.35% (P>0.05), increased serum SOD activity by 40.90% (P<0.05), and decreased serum MDA content by 16.20% (P>0.05). The OO-400 group achieved similar levels in SGR, intestinal structure, serum SOD activity, and MDA content as the CG group.

Under the experimental conditions, the results indicate that supplementation of 0.02% COS in a conventional diet containing 4% soybean oil can promote growth, improve intestinal structure, and enhance non-specific immune capacity of crucian carp. Oxidized soybean oil negatively affects the growth and health of crucian carp, while supplementation of 0.04% COS in such diets can alleviate these negative effects and restore fish health to normal levels.

Keywords: crucian carp (Carassius auratus gibelio); chitosan oligosaccharide; oxidized oil; intestinal structure; non-specific immune indexes

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Chitosan oligosaccharides (COS) are low molecular weight oligosaccharides (<2,000 u) derived from chitin through deacetylation to form chitosan (with molecular weight of hundreds of thousands to millions of Daltons and absorption rate of 1-5%), followed by enzymatic hydrolysis. Comprising 2-10 glucosamine units linked by -1,4 glycosidic bonds, COS represents the only naturally occurring alkaline amino polysaccharide in substantial quantities. As a low molecular weight oligosaccharide product, COS exhibits excellent water solubility, high biological activity, and easy absorption, differing from chitosan in molecular weight, water solubility, and absorbability. The structural characteristics, degree of acetylation, purity, and solubility of oligosaccharides produce different effects. COS supplementation in aquafeed has been reported previously. Su et al. reported that COS promoted growth and enhanced non-specific immune function in tiger pufferfish (Takifugu rubripes). Tian et al. demonstrated that COS improved intestinal structure and modulated major intestinal flora in GIFT tilapia (Oreochromis niloticus). Other studies have shown that COS promotes growth in black tiger shrimp (Penaeus monodon) and enhances antioxidant capacity and resistance to oxidative stress.

Crucian carp (Carassius auratus gibelio) belongs to Cypriniformes, Cyprinidae. It was developed through artificial insemination using female crucian carp from Shuangfeng Reservoir in Fangzeng County, Heilongjiang Province as the maternal parent ( ) and male red carp from Xingguo County, Jiangxi Province as the paternal parent ( ), stimulating gynogenetic development of the eggs. Crucian carp has been widely cultured in Jiangsu, Shandong, and Hubei provinces, representing one of China' s important freshwater aquaculture species and holding a significant position in freshwater aquaculture. With deteriorating culture environments and insufficient research on physiology, nutrition, and diseases, various bacterial, viral, parasitic, and nutritional diseases (oxidized oils) have caused substantial pressure on crucian carp aquaculture, with nutritional diseases posing a serious threat. Maintaining physiological health and enhancing immune defense through dietary approaches represents an important technical challenge. As one of the three major nutrients for fish, oils provide energy and essential fatty acids, constitute important cellular components, and serve as carriers for fat-soluble nutrients to enhance their absorption and utilization. However, unsaturated fatty acids in oils are highly susceptible to oxidation during storage under high temperature, humidity, light, and metal ion conditions, producing various primary and secondary oxidation products such as peroxides, alcohols, aldehydes, ketones, hydrocarbons, esters, and polymers. These oxidation products can disrupt normal physiological and biochemical functions, affect growth and development, induce nutritional diseases, and compromise health when ingested by animals, drawing widespread attention from nutritionists. Therefore, preventing and mitigating the harmful effects of oxidized oils on physiological health and immune defense in aquatic animals is of great significance.

This study used crucian carp as the experimental model to investigate the effects of dietary COS supplementation on growth performance, intestinal structure, and non-specific immune indexes, while examining whether COS could alleviate the side effects caused by oxidized oils in feed.

1.1 Experimental Materials

The COS used in this study was provided by Zhongtaihe (Beijing) Science & Technology Development Company. Produced from aquatic animal shell polysaccharides using enzymatic degradation of chitosan coupled with membrane separation technology, the product contained 10% COS with maltodextrin as carrier, degree of deacetylation >90% (2-amino oligosaccharide), pH 7.0-9.0, molecular weight <2,000 u, and was water-soluble.

1.2 Experimental Diets

Feed ingredients were provided by Jiangsu Dafeng Huachen Aquatic Industry Company. Normal soybean oil was "Fulinmen" brand first-grade soybean oil from COFCO. Oxidized soybean oil was prepared by adding 30 mg/L ferrous sulfate heptahydrate, 15 mg/L copper sulfate pentahydrate, 600 mg/L 30% hydrogen peroxide, and 0.3% water to normal soybean oil. The mixture was placed in a water bath at (80±2)°C, oxygenated for 1 minute every 30 minutes (cycling) for 14 days.

Two basal diets were formulated with either normal or oxidized soybean oil as lipid sources, then supplemented with 0%, 0.02%, 0.04%, and 0.06% COS, resulting in eight experimental diets (CG, OO, CG-200, CG-400, CG-600, OO-200, OO-400, OO-600). The diet without COS containing normal soybean oil (CG) served as the positive control, while the diet without COS containing oxidized soybean oil (OO) served as the negative control. Diet composition and nutrient levels are shown in Table 1 . No significant differences were observed in moisture, crude protein, or crude lipid content among dietary groups ($P>0.05$).

Table 1 Composition and nutrient levels of experimental diets (air-dry basis)

Feed ingredients were ground to pass through a 60-mesh sieve. Major ingredients (>4% proportion) were mixed for 10 minutes, then minor premixes were gradually added and mixed for another 10 minutes. Finally, soybean oil (or oxidized soybean oil) and water (for pelleting) were gradually incorporated (the mixture was passed through a 40-mesh sieve, with particles ground using a crusher) and mixed for 20 minutes. The mixed feed was pelleted using a small ring-die pelletizer at 65°C to produce 1.5 mm diameter pellets (2-3 mm length), air-dried to approximately 13% moisture, stored at -20°C, and naturally thawed before feeding.

1.3 Experimental Fish and Culture Management

The feeding trial was conducted in net cages at Huaken Pond, Jiangsu Dafeng Huachen Aquatic Industry Company. Twenty-four experimental net cages (1.5 m × 1.5 m × 2.0 m) were installed in a 40 m × 60 m pond. Two paddlewheel aerators were placed in the center to ensure uniform dissolved oxygen, supplemented by a microporous aeration blower. A circular microporous aeration disc (diameter 0.5 m) made of nanotube (diameter 20 mm) was installed between every two cages at 1.8 m depth. Aeration equipment was turned off during feeding, with microporous aeration applied 1 hour before feeding and paddlewheel aerators operating continuously otherwise.

Experimental crucian carp were purchased from Jiangsu Dafeng Huachen Aquatic Industry Company and fasted for 24 hours before transport. Nine hundred sixty healthy fish with uniform size (average weight 7.60±0.05 g) were selected, disinfected, and randomly distributed into 8 groups with 3 replicates (net cages) per group, totaling 24 cages with 40 fish per cage. Fish were acclimated for 2 weeks using corresponding control diets before the formal trial. Fish were fed twice daily (06:00-08:30 and 17:00-19:30) at 3-5% of body weight, with body weight estimated every 10 days to adjust feeding rates. The formal feeding period lasted 72 days. Water temperature was recorded at 06:00 and 18:00 daily, and water quality at 30 cm depth was measured every 5 days. During the trial, water temperature ranged 22-36°C, dissolved oxygen >5.0 mg/L, pH 8.2-8.6, ammonia <0.2 mg/L, nitrite <0.01 mg/L, and sulfide <0.05 mg/L.

1.4 Sample Collection

At the end of the trial, fish were fasted for 24 hours before sampling.

1.4.1 Whole Fish Collection Before the trial, 10 fish were randomly sampled as initial samples for whole-body proximate analysis. At the end of the trial, fish in each cage were counted and weighed to calculate survival rate, weight gain rate, specific growth rate, and feed conversion ratio. Two fish per cage were randomly sampled for whole-body proximate analysis.

1.4.2 Serum Collection Blood was collected from the caudal vein using 1 mL sterile syringes, placed in 2 mL Eppendorf tubes, allowed to coagulate naturally for 30 minutes, then centrifuged (4°C, 3,500 r/min) for 15 minutes. Upper serum (200 µL per tube) was collected, mixed, and aliquoted into 0.5 mL Eppendorf tubes (minimum 12 tubes per cage), snap-frozen in liquid nitrogen, and stored at -80°C for non-specific immune index analysis.

1.4.3 Intestinal Tissue Sample Collection Two fish per cage were randomly selected, and approximately 1 cm of mid-intestine was excised, washed in sterile 0.75% saline, and fixed in 10% formalin for histological sectioning. Intestinal segments were sampled from consistent locations across all groups.

1.5 Analysis Methods

1.5.1 Proximate Analysis Samples were freeze-dried to constant weight for moisture determination, then analyzed for crude protein and crude lipid content using national standard methods. Acid value, peroxide value, and malondialdehyde content (in feed and oil) were also determined using standard methods.

1.5.2 Serum Non-Specific Immune Index Analysis Serum superoxide dismutase (SOD) activity, catalase (CAT) activity, and malondialdehyde (MDA) content were determined using assay kits from Nanjing Jiancheng Bioengineering Institute according to manufacturer instructions.

1.5.3 Intestinal Histology Fixed intestinal tissues were processed through washing, alcohol gradient dehydration, clearing, paraffin infiltration, embedding, and sectioning at 5 µm thickness. Sections were stained with hematoxylin-eosin (HE) and observed under optical microscopy. Images were captured using a Nikon COOL-PIX4500 camera and quantified using Smart-4500 software to measure intestinal fold height, intestinal fold width, and intestinal wall thickness.

1.6 Data Processing and Statistical Analysis

Data are expressed as mean ± standard deviation. Statistical analysis was performed using SPSS 22.0 software. Duncan’ s multiple comparison test was applied when significant differences were detected among groups, with significance level set at P<0.05.

2 Results

2.1 Effects of COS on Growth Performance of Crucian Carp

After 72 days of pond net cage culture, growth performance data are presented in Table 2 . No mortality occurred during the trial, with 100% survival in all groups. Compared with the CG group, the SGR of CG-200 group increased by 16.20% (P<0.05), while CG-400 and CG-600 groups showed no significant changes (P>0.05). The OO group exhibited decreased SGR by 8.10% (P<0.05). Compared with the OO group, OO-200, OO-400, and OO-600 groups showed increased SGR by 5.36%, 5.75%, and 4.21%, respectively, but these differences were not significant (P>0.05). The SGR of OO-200, OO-400, and OO-600 groups did not differ significantly from the CG group (P>0.05). The weight gain rate of CG-200 group was significantly higher than other groups (P<0.05), with no significant differences among other groups (P>0.05).

Regarding feed conversion ratio, CG-200 group decreased by 6.33% compared with CG group, but the difference was not significant (P>0.05). CG-400, CG-600, and OO groups increased by 28.58%, 80.38%, and 17.72% compared with CG group, respectively (P<0.05). OO-200, OO-400, and OO-600 groups decreased by 6.45%, 2.15%, and 9.14% compared with OO group, respectively, but these differences were not significant (P>0.05).

Table 2 Effects of COS on growth performance of crucian carp (Carassius auratus gibelio) (n=3)

Weight gain rate = 100×(Wt-W0)/W0; Specific growth rate = 100×(lnWt-lnW0)/t; Feed conversion ratio = Wf/(Wt-W0). Where Wt and W0 represent final and initial average body weight, respectively, and Wf represents total feed weight.

Values in the same row with different letter superscripts indicate significant differences (P<0.05). The same as below.

2.2 Effects of COS on Body Composition of Crucian Carp

As shown in Table 3 , neither COS supplementation nor oil oxidation significantly affected whole-body moisture, crude protein, or crude lipid content (P>0.05).

Table 3 Effects of COS on body composition of crucian carp (Carassius auratus gibelio) (n=3)

2.3 Effects of COS on Intestinal Structure of Crucian Carp

Intestinal sections from the same mid-intestinal location were prepared and observed under microscopy (Figure 1 [Figure 1: see original paper]). Compared with the CG group, the OO group showed decreased intestinal fold height, increased fold width, and thinner intestinal wall. The CG-200 group exhibited neatly arranged intestinal villi with greater fold height and smaller width. The OO-400 group showed improved intestinal fold height, reduced width, and increased wall thickness compared with the OO group, reaching levels comparable to the CG group.

Images 1-8 represent intestinal sections of crucian carp from CG, OO, CG-200, CG-400, CG-600, OO-200, OO-400, and OO-600 groups, respectively. T indicates intestinal wall thickness, H indicates intestinal fold height, and W indicates intestinal fold width.

Fig.1 Intestinal tissue sections of crucian carp (Carassius auratus gibelio)

Microscopic images were measured and quantified using Smart-4500 software to obtain data on intestinal fold height, width, and wall thickness (Figures 2 [Figure 2: see original paper], 3 [Figure 3: see original paper], and 4 [Figure 4: see original paper]).

Figure 2 shows that compared with the CG group, CG-200 group significantly increased intestinal fold height by 86.84% ($P<0.05$), while CG-400, CG-600, and OO groups decreased fold height, with CG-600 and OO groups showing significant reductions of 15.08% and 17.81%, respectively ($P<0.05$). Compared with the OO group, OO-200, OO-400, and OO-600 groups increased fold height by 9.98%, 18.89%, and 4.48%, respectively, with OO-400 group showing significant difference ($P<0.05$). The fold height of OO-200 and OO-400 groups did not differ significantly from the CG group ($P>0.05$).

Figure 3 shows that compared with the CG group, CG-200 and CG-400 groups decreased intestinal fold width without significant differences ($P>0.05$), while CG-600 and OO groups significantly increased width by 47.38% and 70.37%, respectively ($P<0.05$). Compared with the OO group, OO-200, OO-400, and OO-600 groups significantly decreased width by 36.65%, 42.99%, and 33.72%, respectively ($P<0.05$), with no significant differences compared with the CG group ($P>0.05$).

Figure 4 shows that intestinal wall thickness followed similar trends as fold height.

Fig.2 Effects of COS on intestinal fold height of crucian carp (Carassius auratus gibelio) (n=6)

Fig.3 Effects of COS on intestinal fold width of crucian carp (Carassius auratus gibelio) (n=6)

Fig.4 Effects of COS on intestinal wall thickness of crucian carp (Carassius auratus gibelio) (n=6)

2.4 Effects of COS on Serum Non-Specific Immune Indexes of Crucian Carp

As shown in Figure 5 [Figure 5: see original paper], compared with the CG group, CG-200, CG-400, and CG-600 groups showed increased serum SOD activity without significant differences ($P>0.05$), with CG-200 group showing the highest increase (6.59%). The OO group significantly decreased SOD activity by 27.35% ($P<0.05$). Compared with the OO group, OO-200, OO-400, and OO-600 groups significantly increased SOD activity by 26.25%, 40.90%, and 18.15%, respectively ($P<0.05$), with OO-400 group reaching levels comparable to the CG group ($P>0.05$).

Regarding serum MDA content, CG-200 and CG-600 groups decreased while CG-400 group increased, but without significant differences ($P>0.05$). The OO group significantly increased MDA content by 25.72% ($P<0.05$). Compared with the OO group, OO-200, OO-400, and OO-600 groups decreased MDA content by 12.98%, 16.20%, and 27.53%, respectively, without significant differences ($P>0.05$), and showed no significant differences compared with the CG group ($P>0.05$). No significant differences were observed in serum catalase activity among all groups ($P>0.05$).

Fig.5 Effects of COS on serum non-specific immune indexes of crucian carp (Carassius auratus gibelio) (n=3)

3 Discussion

Under the experimental conditions, supplementation of 0.02% COS in the 4% soybean oil diet (conventional diet) increased SGR by 16.20% and decreased feed conversion ratio by 6.33% in crucian carp compared with the CG group. However, higher COS supplementation (0.04% and 0.06%) did not significantly affect SGR. Replacement of normal soybean oil with oxidized soybean oil decreased SGR by 8.10% and increased feed conversion ratio by 17.72%. Compared with the OO group, supplementation of 0.04% COS in the 4% oxidized soybean oil diet increased SGR by 5.75% and decreased feed conversion ratio by 2.15%, restoring growth performance and feed efficiency to levels comparable to the conventional diet.

The mechanisms by which COS affects growth performance and feed efficiency may be attributed to its physicochemical properties, including degree of deacetylation, charge distribution, and chemical modifications. COS influences animal growth through several pathways: (1) promoting mineral absorption via active groups (–NH and –OH) that readily bind mineral elements for intestinal absorption; and (2) improving intestinal structure by increasing villus height and density while decreasing width, thereby enlarging the contact area with food and enhancing digestion and absorption.

Improvement of intestinal mucosal structure represents an important action site of COS. The fish intestine is the primary site for nutrient digestion and absorption and the largest mucosal immune organ, with normal morphological structure being fundamental for nutrient absorption and intestinal immunity. This study found that 0.02% COS supplementation resulted in neatly arranged intestinal villi, increasing intestinal fold height by 86.84% and wall thickness by 20.45% while decreasing fold width by 12.18% compared with the CG group, demonstrating optimal intestinal structure improvement. Oxidized soybean oil damaged intestinal structure, decreasing fold height by 17.81% and wall thickness by 30.33% while increasing fold width by 70.37%. Supplementation of 0.04% COS in oxidized oil diets increased fold height by 18.89%, decreased width by 2.98%, and increased wall thickness by 38.35% compared with the OO group, effectively alleviating the negative effects of oxidized oil and restoring intestinal structure to normal levels. These results indicate that appropriate COS supplementation can effectively improve intestinal structure and increase nutrient absorption area. Similar findings were reported by Dimitroglou et al. in gilthead sea bream (Sparus aurata) and Pryor et al. in Gulf of Mexico sturgeon fed mannan oligosaccharides.

The intestinal structure improvement by COS may involve inhibition of pathogen colonization on intestinal mucosa, modulation of intestinal flora, and promotion of intestinal epithelial cell proliferation, through two main pathways: (1) COS provides high-affinity ligands that offer specific binding sites for bacterial lectins, thereby preventing pathogen binding to intestinal epithelial cells; and (2) COS promotes proliferation of beneficial bacteria, which more extensively attach to the intestinal surface and reduce contact between harmful bacteria and the intestinal wall. Additionally, COS may enhance nutrient absorption by serving as a growth factor for beneficial bacteria, producing B vitamins, and promoting intestinal peristalsis. This study found increased intestinal wall thickness after COS supplementation, which differs from Liu et al.' s observation of decreased wall thickness in Nile tilapia (Oreochromis niloticus × O. aureus), possibly due to differences in species, region, culture period, and water conditions requiring further investigation.

As water-soluble, easily absorbed 2-amino oligosaccharides, COS may be absorbed by fish and exert physiological functions in antioxidant defense and maintenance of immune system integrity, thereby enhancing immune capacity. Fish rely primarily on non-specific immunity as lower vertebrates. Serum SOD activity, catalase activity, and MDA content are important indicators of fish non-specific immunity. SOD is a crucial antioxidant enzyme that scavenges free radicals; catalase is a key enzyme in biological defense that eliminates hydrogen peroxide; and MDA is the end product of lipid peroxidation that causes cellular damage, with blood MDA content indirectly reflecting cellular damage extent. This study showed that 0.02% COS supplementation increased serum SOD activity by 6.59% and decreased MDA content by 16.94% compared with the CG group. Oxidized soybean oil decreased SOD activity by 27.35% and increased MDA content by 25.72%. Supplementation of 0.04% COS in oxidized oil diets increased SOD activity by 40.90% and decreased MDA content by 16.20% compared with the OO group, restoring immune parameters to normal levels. The enhanced non-specific immunity by COS may be achieved through: (1) direct hydroxyl radical (·OH) scavenging capacity that improves antioxidant capacity and protects immune organs; and (2) promoting proliferation of beneficial bacteria such as Bifidobacterium that enhance antibody levels and activate macrophage phagocytic activity.

Higher COS supplementation does not necessarily produce better effects in crucian carp, as CG-400 and CG-600 groups showed significantly reduced SGR compared with CG-200 group. Possible reasons include: (1) COS is not digested in the intestine but directly absorbed by intestinal epithelial cells into blood circulation, where it binds various groups to exert multiple functions. With increasing supplementation, fish cannot regulate COS absorption, and although the increase is modest, excessive COS in blood circulation may over-bind other groups and disrupt homeostasis; and (2) while appropriate COS supplementation enhances immunity, excessive amounts may cause immunosuppression and affect the immune system.

Oxidized oils in feed negatively affect crucian carp growth, and COS supplementation can partially repair these effects. Oxidized oils damage growth performance and intestinal health in aquatic animals through several mechanisms: (1) reduced palatability and feed intake; (2) primary and secondary oxidation products such as peroxides and MDA disrupt free radical metabolism balance, increase abnormal free radicals, destroy antioxidant enzyme activity, and cause oxidative stress damage; and (3) oxidized oils cause empty gastrointestinal tracts with water accumulation in rainbow trout (Oncorhynchus mykiss) and open tight junction structures with increased intestinal permeability in grass carp (Ctenopharyngodon idella). The rich biological activities of COS, particularly its antioxidant properties, may be the main reason for promoting growth and alleviating negative effects of oxidized soybean oil. The COS used in this study had molecular weight $<$2,000 u and degree of deacetylation $>$90%. Feng et al. compared antioxidant capacity of water-soluble COS with different molecular weights, finding that lower molecular weight conferred stronger antioxidant capacity, possibly due to intermolecular hydrogen bonding effects. High molecular weight chitosan has compact structure with strong intramolecular hydrogen bonds that limit antioxidant capacity, while low molecular weight chitosan has looser structure with weaker hydrogen bonds and more free hydroxyl and amino groups, resulting in superior superoxide anion radical scavenging capacity. Je et al. investigated radical scavenging capacity of COS with different deacetylation degrees, finding that higher deacetylation conferred better radical scavenging ability, likely because COS provides positive electrons to react with radicals and convert them into more stable products, terminating radical chain reactions. These findings suggest that the COS used in this study possesses strong antioxidant capacity that can improve crucian carp antioxidant ability and alleviate damage caused by oxidized soybean oil.

Conclusions: 1. Supplementation of 0.02% COS in conventional diets containing 4% soybean oil can promote growth and maintain fish health in crucian carp. 2. Oxidized soybean oil negatively affects growth and health of crucian carp, and supplementation of 0.04% COS in such diets can alleviate these negative effects, restoring fish health to levels comparable to conventional diets.

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