Effects of Xylooligosaccharides on Growth Performance, Serum Biochemical and Immune Indices, and Intestinal Microbiota Composition in Juvenile Japanese Seabass (Lateolabrax japonicus): Postprint

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Abstract

This study aimed to investigate the effects of xylo-oligosaccharides (XOS) on growth performance, serum biochemical and immune parameters, and intestinal microbiota composition in juvenile Japanese sea bass (Lateolabrax japonicus). A total of 360 juvenile Japanese sea bass with uniform size [(19.37±0.196) g] were randomly allocated into 6 groups, each consisting of 3 replicates with 20 fish per replicate. The groups were fed diets supplemented with 0 (control), 200 (D200), 400 (D400), 600 (D600), 800 (D800), and 1000 mg/kg xylo-oligosaccharides (D1000) in the basal diet for 45 days. The results demonstrated: 1) Compared with the control group, the weight gain rate (WGR) and specific growth rate (SGR) of D200, D400, and D600 groups increased ($P>0.05$), while those of D800 ($P>0.05$) and D1000 groups ($P<0.05$) decreased. 2) Serum total protein content was significantly increased in D200, D400, D600, D800, and D1000 groups ($P<0.05$); serum high-density lipoprotein cholesterol content was significantly increased in D400 and D800 groups ($P<0.05$); and serum total cholesterol content was significantly decreased in D200 group ($P<0.05$). 3) Serum superoxide dismutase activity was significantly decreased in D400, D600, D800, and D1000 groups ($P<0.05$); serum catalase activity was significantly decreased in D200 and D400 groups ($P<0.05$); serum lysozyme activity was significantly increased in D400 and D800 groups ($P<0.05$); and serum malondialdehyde content in D200 group decreased ($P>0.05$). 4) Intestinal Salmonella counts were significantly decreased in D200, D400, D600, D800, and D1000 groups ($P<0.05$); intestinal Escherichia coli counts were significantly decreased in D200, D400, and D1000 groups ($P<0.05$); and intestinal Bifidobacterium counts were significantly increased in D200, D400, and D600 groups ($P<0.05$). In conclusion, comprehensive evaluation of all experimental indices indicated that dietary supplementa-

tion of 350–560 mg/kg xylo-oligosaccharides exerted optimal effects on growth performance, immune function, and intestinal health in juvenile Japanese sea bass.

Full Text

Abstract

This study was conducted to determine the effects of xylooligosaccharide (XOS) on growth performance, serum biochemical and immune indices, and intestinal microflora composition of juvenile Japanese seabass (*Lateolabrax japonicus*). A total of 360 juvenile Japanese seabass with uniform size [(19.37±0.196) g] were randomly assigned to 6 groups with 3 replicates per group and 20 fish per replicate. Fish in the 6 groups were fed the basal diets supplemented with 0 (control group), 200 (D200 group), 400 (D400 group), 600 (D600 group), 800 (D800 group), and 1,000 mg/kg XOS (D1000 group), respectively. The experiment lasted for 45 days. The results showed as follows: 1) compared with the control group, the weight gain rate (WGR) and specific growth rate (SGR) of D200, D400, and D600 groups were increased ($P>0.05$), while the WGR and SGR of D800 ($P>0.05$) and D1000 groups ($P<0.05$) were decreased. 2) Compared with the control group, the serum total protein content of D200, D400, D600, D800, and D1000 groups was significantly increased ($P<0.05$), the serum high-density lipoprotein cholesterol content of D400 and D800 groups was significantly increased ($P<0.05$), and the serum total cholesterol content of D200 group was significantly decreased ($P<0.05$). 3) Compared with the control group, the serum superoxide dismutase activity of D400, D600, D800, and D1000 groups was significantly decreased ($P<0.05$), the serum catalase activity of D200 and D400 groups was significantly decreased ($P<0.05$), the serum lysozyme activity of D400 and D800 groups was significantly increased ($P<0.05$), and the serum malondialdehyde content of D200 group was decreased ($P>0.05$). 4) Compared with the control group, the intestinal *Salmonella* number of D200, D400, D600, D800, and D1000 groups was significantly decreased ($P<0.05$), the intestinal *Escherichia coli* number of D200, D400, and D1000 groups was significantly decreased ($P<0.05$), and the intestinal *Bifidobacterium* number of D200, D400, and D600 groups was significantly increased ($P<0.05$). In conclusion, based on comprehensive evaluation of all experimental indices, dietary supplementation of 350–560 mg/kg XOS had the best effects on growth performance, immune function, and intestinal health of juvenile Japanese seabass.

Keywords: *Lateolabrax japonicus*; growth performance; xylooligosaccharide; non-specific immunity; intestinal microflora

Japanese seabass (*Lateolabrax japonicus*), also known as spotted sea bass, belongs to the class Osteichthyes, order Perciformes, family Serranidae, and genus *Lateolabrax*. It is mainly distributed along the coasts of the Korean Peninsula, China, and Vietnam. Japanese seabass is eurythermal and euryhaline with delicious meat, making it widely cultured. As a major aquaculture species in China, its safety has received extensive attention. With the development of intensive aquaculture, the abuse of antibiotics and other drugs has led to problems such as drug resistance and residues. Many researchers are committed to finding alternatives to antibiotics. Prebiotics are not digested by digestive enzymes, but can be utilized by beneficial intestinal bacteria after fermentation in the gut. The resulting metabolites and micronutrients provide energy and nutrition for the organism, lower intestinal pH, and promote the proliferation of beneficial bacteria while inhibiting harmful bacteria. Currently, some prebiotics such as fructooligosaccharides and mannan oligosaccharides have been widely used as growth promoters in poultry diets to replace antibiotics. Xylooligosaccharide (XOS), also known as wood oligosaccharide, is obtained through enzymatic hydrolysis of xylan by xylanase, primarily consisting of xylobiose and xylotriose. XOS can promote the proliferation of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* in the intestine [1-4]. Studies have shown that XOS is more effective than other oligosaccharides in increasing *Bifidobacterium* numbers [5]. Additionally, XOS can be widely sourced from inexpensive agricultural by-products rich in xylan, such as corn cobs, bagasse, and cottonseed hulls [6]. Due to its unique properties, XOS has attracted considerable attention as a prebiotic for improving animal health and growth performance. Research indicates that XOS promotes growth, enhances immunity, and improves intestinal microflora in aquatic animals such as sea cucumbers, European sea bass, grass carp, whitefish, and Nile tilapia [7-11]. Therefore, this study investigated the effects of different XOS levels on juvenile Japanese seabass by examining growth performance, serum biochemical and immune indices, and intestinal microflora composition, providing a theoretical basis for the application of XOS as a green additive in aquafeeds.

1.1 Experimental Design and Basal Diet

Based on the basic nutritional requirements of juvenile Japanese seabass, a basal diet was formulated using fish meal (crude protein content 74.35%, crude fat content 8.43%) and soybean meal (crude protein content 48.25%, crude fat content 1.06%) as the main protein sources, and fish oil and soybean oil as the main lipid sources. Wheat flour was used to balance the formulation and maintain total diet balance. The composition and nutrient levels of the basal diet are shown in Table 1. A single-factor experimental design was adopted. A total of 360 healthy juvenile Japanese seabass with uniform size [(19.37±0.196) g] were randomly assigned to 6 groups with 3 replicates per group and 20 fish per replicate. Fish in each group were fed diets supplemented with 0 (control group), 200 (D200 group), 400 (D400 group), 600 (D600 group), 800 (D800 group), and 1,000 mg/kg XOS (D1000 group), respectively. XOS was purchased from Jiangsu Kangwei Biological Co., Ltd. All ingredients were ground to pass through a 60-mesh sieve, mixed uniformly, and processed into 2.5 mm pellets using a small extruder. The pellets were dried in a 55 ℃ oven, naturally cooled, and stored at -20 ℃.

1.2 Experimental Fish Acclimation

The feeding trial was conducted at the Marine Experimental Station of Jimei University. Juvenile Japanese seabass (purchased from Jinxing Hatchery in Zhangpu County, Fujian Province) were temporarily reared in circulating filtration barrels (1,200 L). During the acclimation period, fish were fed self-made feed and gradually acclimated to pure freshwater culture. After two weeks of acclimation in freshwater, the experimental fish were ready for the trial.

1.3 Feeding Management

After acclimation, 360 healthy juvenile Japanese seabass with uniform size were randomly distributed into 18 glass aquaria (80 cm × 45 cm × 45 cm) in a recirculating water system, with 20 fish per aquarium. During the experimental period, fish were fed twice daily at 08:00–08:30 and 18:00–18:30. Residual feed and feces were promptly collected, and water was exchanged twice daily (1/3 of the total volume each time). The experiment lasted for 45 days. Throughout the trial, continuous aeration was provided to maintain dissolved oxygen 7 mg/L, water temperature at (28±1) ℃, and pH at 7.3–8.1. Daily observations were made on feeding behavior and mortality, with records kept promptly.

1.4 Sample Collection and Processing

After 45 days of culture, fish were fasted for 24 hours. Fish were anesthetized with eugenol cement, and the total weight and number of fish in each tank were measured. Five fish were randomly selected from each tank to measure body weight and length. Blood was collected from the caudal vein using a 1 mL sterile syringe and placed in 1.5 mL centrifuge tubes. After standing at 4 ℃ for 12 hours, serum was collected by centrifugation at 4 ℃ and 3,500 r/min for 10 minutes. Serum from these five fish was pooled and stored at -80 ℃ for subsequent serum biochemical and immune index analysis. Another 10 fish were randomly selected and dissected to quickly remove the intestine (surface fat was removed with physiological saline). Intestinal tissue from five fish was snap-frozen in liquid nitrogen and stored at -80 ℃ for intestinal digestive enzyme analysis. The intestines from the remaining five fish were ligated at both ends with fine string and brought back to the laboratory for intestinal content collection under aseptic conditions to determine intestinal flora composition.

1.5.1 Growth Performance Indicators and Calculation Formulas

Weight gain rate (WGR, %) = 100 × (Wt - W0) / W0
Specific growth rate (SGR, %/d) = 100 × [ln(Wt) - ln(W0)] / d
Survival rate (SR, %) = 100 × Nt / N0
Condition factor (CF, %) = 100 × Wt / L^3
Feed conversion ratio (FCR, %) = 100 × (dry weight of feed fed - dry weight of

residual feed) / (Wt - W0)

Where: W0 = initial body weight (g); Wt = final body weight (g); d = culture days; N0 = initial number of fish; Nt = final number of surviving fish; L = body length (cm).

1.5.2 Serum Biochemical and Immune Index Determination

Serum biochemical and immune indices included: triglycerides (TG), total cholesterol (TC), superoxide dismutase (SOD), alkaline phosphatase (AKP), malondialdehyde (MDA), total protein (TP), catalase (CAT), high-density lipoprotein cholesterol (HDL-C), and lysozyme (LZM). All these indices were measured using assay kits provided by Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions. The main instruments used were a microplate reader (Biotek) and a UV-1200 UV-Vis spectrophotometer.

1.5.3 Intestinal Flora Composition Determination

Intestinal content (0.5 g) was weighed and mixed with 4.5 mL sterile physiological saline at a 1:9 mass-to-volume ratio. After vortexing and centrifugation, a 10^{1} stock solution was prepared. Then 0.5 mL of the supernatant was added to 4.5 mL sterile physiological saline for 10-fold serial dilution up to 10 . Each dilution gradient was performed in triplicate, with 100 L of bacterial suspension plated per plate. Anaerobic bacteria were cultured under anaerobic conditions in a self-made anaerobic chamber (containing Mitsubishi AnaeroPack™-Anaero gas-generating bags) for 72 hours. After incubation, plates with 30–300 colonies were selected for counting. Results were expressed as log colony-forming units per gram of intestine [lg(CFU/g)]. All culture media were purchased from Qingdao High-tech Industrial Park Hope Bio-Technology Co., Ltd.

1.6 Statistical Analysis

All experimental data were analyzed using SPSS 19.0 software for one-way ANOVA. If significant differences were detected, Duncan' s multiple comparison test was applied. Differences were considered significant at $P < 0.05$. Results are presented as mean ± standard deviation (mean±SD).

2.1 Effects of XOS on Growth Performance of Juvenile Japanese Seabass

As shown in Table 2, compared with the control group, the WGR and SGR of D200, D400, D600, D800, and D1000 groups showed a trend of initial increase followed by decrease. The WGR and SGR of D200, D400, and D600 groups were increased ($P>0.05$), while those of D800 ($P>0.05$) and D1000 groups ($P<0.05$) were decreased. There were no significant differences in FCR, CF, and SR among all groups ($P>0.05$).

The relationship between weight gain rate (Y, %) and XOS supplementation level (X, mg/kg) was calculated by regression equation:
$Y = -1.913\times10\ X^2 + 0.13422X + 263.271$ ($R^2 = 0.604$).
Based on this formula, the maximum weight gain rate was achieved at an XOS supplementation level of 350.8 mg/kg.

2.2 Effects of XOS on Serum Biochemical Indices of Juvenile Japanese Seabass

As shown in Table 3, compared with the control group, the serum TP content of D200, D400, D600, D800, and D1000 groups was significantly increased ($P<0.05$). The serum TC content of D200 ($P<0.05$) and D400 groups ($P>0.05$) was decreased, while that of D600 ($P>0.05$), D800 ($P<0.05$), and D1000 groups ($P>0.05$) was increased. The serum TG content of D200 ($P>0.05$) and D400 groups ($P<0.05$) was decreased, while that of D600 ($P>0.05$), D800 ($P<0.05$), and D1000 groups ($P>0.05$) was increased. The serum HDL-C content of D200 ($P>0.05$), D400 ($P<0.05$), D600 ($P>0.05$), D800 ($P<0.05$), and D1000 groups ($P>0.05$) was increased.

2.3 Effects of XOS on Serum Immune Indices of Juvenile Japanese Seabass

As shown in Table 4, compared with the control group, the serum SOD activity of D200 ($P>0.05$), D400 ($P<0.05$), D600 ($P<0.05$), D800 ($P<0.05$), and D1000 groups ($P<0.05$) was decreased. The serum CAT activity of D200 ($P<0.05$), D400 ($P<0.05$), D600 ($P>0.05$), D800 ($P>0.05$), and D1000 groups ($P>0.05$) was decreased. The serum LZM activity of D200 ($P>0.05$), D400 ($P<0.05$), D600 ($P>0.05$), D800 ($P<0.05$), and D1000 groups ($P>0.05$) was increased. The serum MDA content of D200 group was decreased ($P>0.05$), while that of D400 ($P<0.05$), D600 ($P>0.05$), D800 ($P<0.05$), and D1000 groups ($P<0.05$) was increased. There were no significant differences in serum AKP activity among all groups ($P>0.05$).

The relationship between serum LZM activity (Y, U/mL) and XOS supplementation level (X, mg/kg) was calculated by regression equation:
$Y = -2.139\times10\ X^2 + 0.23959X + 240.755$ ($R^2 = 0.246$).
Based on this formula, the optimal serum LZM activity was achieved at an XOS supplementation level of 560 mg/kg.

2.4 Effects of XOS on Intestinal Microflora Composition of Juvenile Japanese Seabass

As shown in Table 5, compared with the control group, the intestinal *Salmonella* number of D200, D400, D600, D800, and D1000 groups was significantly decreased ($P<0.05$). The intestinal *Escherichia coli* number of D200 ($P<0.05$), D400 ($P<0.05$), D600 ($P>0.05$), and D1000 groups ($P<0.05$) was decreased, while that of D800 group was significantly increased ($P<0.05$). The intestinal *Bifidobacterium* number showed a trend of initial increase followed by decrease with increasing XOS supplementation. The intestinal *Bifidobacterium* number of D200, D400, and D600 groups was significantly increased ($P<0.05$), while that of D800 and D1000 groups was decreased ($P>0.05$).

The relationship between intestinal *Bifidobacterium* number [Y, lg(CFU/g)] and XOS supplementation level (X, mg/kg) was calculated by regression equation:
$Y = -1.9\times10\ \ X^2 + 1.65\times10\ {}^3X + 8.041$ ($R^2 = 0.631$).
Based on this formula, the maximum intestinal *Bifidobacterium* number was achieved at an XOS supplementation level of 434.2 mg/kg.

3.1 Effects of XOS on Growth Performance and Intestinal Microflora Composition of Juvenile Japanese Seabass

As a prebiotic, XOS has been reported to promote growth. In this study, WGR of juvenile Japanese seabass was improved when XOS supplementation was 200-600 mg/kg, which is consistent with studies by Li et al. [12] on turbot and Xu et al. [13] on gibel carp. However, when supplementation exceeded 800 mg/kg, an inhibitory effect on WGR was observed, which differs from the results of Li et al. [12] and Xu et al. [13]. Possible reasons for these discrepancies include: 1) As a non-nutritive substance, excessive XOS may act as an anti-nutritional factor. Moreover, different feed compositions can yield different results. Guerreiro et al. [9] reported that 1% XOS supplementation in European sea bass diets promoted growth when plant protein sources were used but inhibited growth when fish meal was the main protein source. 2) Oligosaccharides promote beneficial intestinal bacteria. In this study, intestinal *Bifidobacterium* number showed a trend of initial increase followed by decrease, and whether excessive supplementation reduces beneficial bacteria requires further investigation. 3) There are substantial differences in intestinal tissue and microflora structure among animals, including variations in digestive tract length, digestive juice properties, and dominant intestinal bacteria. To date, reports on XOS in aquatic animals are limited, and its growth-promoting effects on Japanese seabass require further study.

Salmonella and *E. coli* are pathogenic bacteria that seriously threaten human and animal health. These harmful bacteria can easily contaminate feed during production and colonize the fish intestine after consumption. Studies have shown that gibel carp infected with *Salmonella* develop intestinal congestion and liver hypertrophy, leading to death [14]. *Bifidobacterium* is a well-known beneficial bacterium that plays an important role in promoting absorption and improving intestinal environment. As a functional oligosaccharide, XOS cannot be digested or absorbed in the animal intestine but can be fermented and utilized by beneficial bacteria, promoting *Bifidobacterium* proliferation while inhibiting harmful bacteria [15-16]. This study demonstrated that XOS supplementation reduced intestinal *Salmonella* and *E. coli* numbers while increasing *Bifidobacterium* numbers, with the best effects observed at 200 and 400 mg/kg supplementation. Human consumption of XOS can increase intestinal *Bifidobacterium* numbers [17]. Petersen et al. [18] reported that fructooligosaccharides and XOS effectively increased intestinal *Bifidobacterium* numbers in mice and inhibited *Salmonella* growth. Our results are consistent with these findings, showing that XOS effectively increased intestinal *Bifidobacterium* numbers in juvenile Japanese seabass while inhibiting *Salmonella* and *E. coli* growth. As shown in Tables 3 and 6, XOS supplementation at 200–600 mg/kg had the best effect on improving intestinal microflora of juvenile Japanese seabass, corresponding to the highest WGR. The data from this study indicate a positive correlation between growth performance and intestinal microflora improvement in juvenile Japanese seabass. This may be because XOS fermentation by beneficial bacteria in the intestine produces short-chain fatty acids and lowers intestinal pH [19], providing energy for intestinal mucosal cells, promoting cell metabolism and growth, and preventing intestinal dysfunction [20], thereby enhancing nutrient absorption and utilization by intestinal mucosa. Low pH environment inhibits harmful bacteria but promotes proliferation of beneficial bacteria such as *Bifidobacterium*. Additionally, low pH facilitates calcium dissolution and absorption. Zafar et al. [21] confirmed that oligosaccharides can increase calcium bioavailability and retention. Non-digestible oligosaccharides can improve mineral absorption and bone formation, exerting certain effects on bone metabolism [22]. Therefore, appropriate XOS supplementation not only promotes growth performance and improves intestinal microecology but also promotes *Bifidobacterium* proliferation and reduces *E. coli* and *Salmonella* numbers, greatly improving meat safety and reducing the risk of human infection from consuming aquatic products contaminated with *E. coli* and *Salmonella*.

3.2 Effects of XOS on Serum Immune Indices of Juvenile Japanese Seabass

Non-specific immunity plays an important role in fish immune defense. SOD is a natural scavenger of oxygen free radicals in organisms. LZM primarily destroys bacterial cell walls, and its enhanced activity indicates strengthened phagocytic activity of macrophages and polymorphonuclear leukocytes. MDA is one of the major harmful substances in lipid peroxidation, indirectly reflecting the degree of cellular damage. Pang et al. [23] reported that XOS effectively increased serum SOD and LZM activities in grass carp. XOS decreased serum SOD, CAT, and AKP activities in juvenile turbot [24]. Xu et al. [25] reported that mannan oligosaccharide decreased serum MDA content in gibel carp. European sea bass fed diets supplemented with 1% XOS showed inhibited SOD and CAT activities in the liver [9]. XOS improved immune function in grass carp and carp, particularly enhancing serum LZM activity [23,26]. Our results are generally consistent with these studies. This study found a negative correlation between serum MDA content and SOD activity. Increased serum MDA content indicates increased aging free radicals, leading to decreased serum SOD activity. The serum MDA content in D200 group was lower than that in the control group, suggesting that low-dose XOS can reduce serum MDA content, though this requires further verification. This study demonstrated that XOS supplementation effectively increased serum LZM activity, reflecting enhanced phagocytic activity of immune cells. Therefore, the enhancement of non-specific immunity in juvenile Japanese seabass by XOS in this study was primarily achieved through increased serum LZM activity.

3.3 Effects of XOS on Serum Biochemical Indices of Juvenile Japanese Seabass

Changes in blood lipid levels reflect lipid metabolism in fish. The main function of HDL-C is to transport cholesterol esters from blood to the liver, and higher levels are beneficial to organism health. Serum TP originates from liver synthesis and intestinal absorption. Studies have shown that TP is crucial for animal growth and development, and its level can reflect the immune stress status of the organism, with serum TP content decreasing in fish under stress [27-29]. XOS supplementation decreased serum TG and TC contents in European sea bass [9]. Gobinath et al. [30] demonstrated that 10% XOS supplementation decreased cholesterol levels in rats. XOS also effectively decreased serum TG content in broilers [31]. Yang et al. [32] showed that chitosan oligosaccharide increased serum TP content in weaned piglets. Huang et al. [33] reported that chitosan oligosaccharide effectively increased serum HDL-C content in juvenile *Jian* carp. In this study, XOS supplementation increased serum TP and HDL-C contents. When supplementation was below 600 mg/kg, serum TG and TC contents decreased, consistent with the above studies. However, when supplementation exceeded 600 mg/kg, serum TG and TC contents were higher than those in the control group, indicating that low-dose XOS can decrease serum TG and TC contents. This may be because low-dose XOS promotes proliferation of beneficial intestinal bacteria, lowers intestinal pH, promotes cholesterol excretion, and reduces blood cholesterol content. Increased serum TP and HDL-C contents also promote lipid reduction.

4 Conclusion

1) Supplementation of 200-600 mg/kg XOS improved growth performance, inhibited serum SOD and CAT activities, and increased LZM activity in juvenile Japanese seabass.

2) Supplementation of 200-400 mg/kg XOS improved blood lipid profiles, decreased intestinal *Salmonella* and *E. coli* numbers, and increased *Bifidobacterium* numbers in juvenile Japanese seabass.

3) Based on comprehensive evaluation of all indices and linear regression analysis, the recommended XOS supplementation level is 350-560 mg/kg.

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