Effects of Dietary Xylo-oligosaccharide Supplementation on Growth Performance, Intestinal Digestive Enzyme Activity, and Immunity of Juvenile Sea Cucumber (Apostichopus japonicus) Postprint

Authors: Li Junhua, Liu Jialiang, Cao Xuebin, Li Jing, Liu Shilin, Zhang Tao

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Abstract

The effects of dietary xylo-oligosaccharides (XOS) on growth performance, intestinal digestive enzyme activities, and immunity of juvenile sea cucumber Apostichopus japonicus Selenka were studied in 120 L blue plastic-steel tanks at a water temperature of 14.8~18.6 ℃ and salinity of (29±1) PSU. Six experimental diets were formulated with XOS supplementation levels of 0 (control), 0.015%, 0.030%, 0.045%, 0.060%, and 0.075%, and were fed to juvenile sea cucumbers with an average body weight of (6.80±1.05) g for 75 d. Each diet was randomly assigned to three tanks, with 45 juveniles stocked per tank. The results showed that dietary XOS supplementation significantly increased the specific growth rate (SGR) of juvenile sea cucumbers ($P<0.05$). The regression equation between dietary XOS supplementation level (X) and SGR of juvenile sea cucumbers (Y) was Y=-151.68X2+13.302X+0.860 7(R2=0.927 5), from which the optimal XOS supplementation level for maximum SGR was determined to be 0.044%. Dietary XOS supplementation had no significant effect on the survival rate (SR) and gutted body wall ratio (GBWR) of juvenile sea cucumbers ($P>0.05$). Dietary XOS supplementation increased the apparent digestibility of dry matter (ADDM) in juvenile sea cucumbers, but only the 0.030% group was significantly higher than the control group ($P<0.05$). Dietary XOS supplementation at 0.030%~0.060% significantly increased intestinal protease and amylase activities, as well as superoxide dismutase (SOD) activity in the coelomic fluid of sea cucumbers ($P<0.05$), with the 0.045% group showing the highest intestinal protease and amylase activities, and the 0.060% group exhibiting the maximum SOD activity in coelomic fluid. At 30 d of feeding, dietary XOS supplementation at 0.030%~0.075% significantly increased alkaline phosphatase (AKP) activity in the coelomic fluid of sea cucumbers ($P<0.05$),

whereas at 60 d of feeding, no significant differences in coelomic fluid AKP activity were observed among groups except for the 0.045% group (P>0.05). It was concluded that dietary XOS supplementation at 0.030%~0.060% could improve intestinal digestive enzyme activities and enhance immunity, thereby promoting the growth of juvenile sea cucumbers; the optimal dietary XOS supplementation level for A. japonicus was 0.044%, and the recommended feeding duration was approximately 2 months.

Full Text

Effects of Dietary Xylo-Oligosaccharides on Growth Performance, Intestinal Digestive Enzyme Activities and Immunity of Juvenile Sea Cucumber (Apostichopus japonicus Selenka)

Li Junhua[1], Liu Jialiang[1], Cao Xuebin[1]*, Li Jing[1], Liu Shilin[2], Zhang Tao[2]

[1]National Algae and Sea Cucumber Engineering Technology Research Center, Shandong Oriental Ocean Sci-Tech Co., Ltd., Yantai 264003, China
[2]Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China

Abstract

A feeding trial was conducted to investigate the effects of dietary xylo-oligosaccharides (XOS) on growth performance, intestinal digestive enzyme activities, and immunity of juvenile sea cucumber (*Apostichopus japonicus* Selenka). Six experimental diets were formulated with XOS supplementation levels of 0 (control), 0.015%, 0.030%, 0.045%, 0.060%, and 0.075%, and fed to juvenile sea cucumbers with an average body weight of (6.80±1.05) g for 75 days at a water temperature of 14.8–18.6 ℃ and salinity of (29±1) PSU. Each diet was randomly assigned to three 120 L blue plastic tanks stocked with 45 juveniles per tank. The results demonstrated that dietary XOS supplementation significantly increased the specific growth rate (SGR) of juvenile sea cucumbers (P<0.05). The regression equation between XOS supplemental level (X) and SGR (Y) was $Y=-151.68X^2+13.302X+0.8607$ ($R^2=0.9275$), indicating that the optimal XOS supplementation level for maximal SGR was 0.044%. No significant effects of XOS were observed on survival rate (SR) or gutted body weight rate (GBWR) (P>0.05). XOS supplementation improved the apparent digestibility of dry matter (ADDM), with only the 0.030% group showing significantly higher values than the control (P<0.05). Diets supplemented with 0.030%–0.060% XOS significantly enhanced intestinal protease and amylase activities as well as superoxide dismutase (SOD) activity in coelomic fluid (P<0.05), with peak protease and amylase activities observed in the 0.045% group and maximal SOD activity in the 0.060% group. After 30 days of feeding, XOS supplementation at 0.030%–0.075% significantly increased alkaline phosphatase (AKP) activity in coelomic fluid (P<0.05), whereas after

60 days, only the 0.045% group maintained significantly elevated AKP activity compared to the control ($P<0.05$). These findings indicate that dietary XOS supplementation at 0.030%–0.060% can enhance intestinal digestive enzyme activities and immunity, thereby promoting growth in juvenile sea cucumbers. The optimal dietary XOS supplementation level is 0.044%, with a recommended feeding duration of approximately two months.

Keywords: xylo-oligosaccharides; sea cucumber (*Apostichopus japonicus* Selenka); growth performance; apparent digestibility; digestive enzyme; immunity

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Sea cucumber (*Apostichopus japonicus* Selenka) is a highly valued marine product with significant nutritional and health benefits, representing an important aquaculture species in China. However, with the expansion of sea cucumber farming, disease outbreaks have become increasingly prevalent, causing substantial economic losses. While antibiotics can address these issues, their long-term or indiscriminate use inevitably leads to bacterial resistance, compromised immune function, drug residues, and environmental pollution. Consequently, research has shifted toward dietary immunostimulants, with polysaccharides emerging as ideal antibiotic alternatives and becoming a focal point in animal nutrition research [1-8].

Xylo-oligosaccharides (XOS) exhibit excellent stability, safety, acid and heat resistance, low effective dosage, and abundant availability. Although XOS cannot be directly digested and absorbed by animals, they can be utilized by beneficial intestinal microorganisms. XOS promotes the proliferation of probiotics such as *Bifidobacterium*, inhibits pathogen growth, and improves nutrient utilization efficiency, earning recognition as the most stable and effective prebiotic for bifidobacterial proliferation. XOS has been widely applied in livestock farming and aquaculture, including shrimp, fish, and sea cucumber production [5-8]. This study aimed to investigate the effects of varying dietary XOS levels on growth performance, digestive enzyme activities, and immunity of juvenile sea cucumbers, determine the optimal XOS supplementation level, and provide theoretical guidance for healthy sea cucumber aquaculture and disease prevention.

1.1 Experimental Diets

The experimental diets consisted of a basal feed mixed with sea mud at a 1:1 ratio prior to feeding. The basal feed was a commercial formula produced by Shandong Oriental Ocean Sci-Tech Co., Ltd., containing macroalgae meal, shell powder, degummed kelp meal, fermented soybean meal, wheat middlings, and yeast, with 19.84% crude protein and 4.28% crude lipid. XOS (35% active ingredient) was provided by Jiangsu Kangwei Biological Co., Ltd. and added to the basal feed at levels of 0 (control), 0.015%, 0.030%, 0.045%, 0.060%, and 0.075%. All ingredients were ultrafine-ground and thoroughly mixed through stepwise expansion to produce a homogeneous powder mixture. The composition and nutrient levels of the basal diet are presented in Table 1 .

1.2 Experimental Animals and Husbandry

Juvenile sea cucumbers with an average body weight of (6.80±1.05) g were obtained from the sea cucumber production facility at Shandong Oriental Ocean Sci-Tech Co., Ltd. and cultured in 120 L blue plastic tanks at a density of 45 individuals per tank, with three tanks randomly assigned to each dietary group. Prior to the experiment, sea cucumbers were acclimated for one week using the control diet without XOS, followed by a 2-day starvation period. Culture water salinity was maintained at (29±1) PSU, temperature ranged from 14.8 to 18.6 ℃, and continuous aeration was provided. Approximately 50% of the water volume was exchanged daily, during which feces were collected and residual feed was removed. After water exchange, fresh seawater was added before feeding. The feed mixture (basal feed and sea mud at 1:1 ratio) was passed through 100-mesh silk screen prior to feeding, with a daily ration of 5% of body weight adjusted according to actual feeding response. The experimental duration was 75 days.

1.3 Sample Collection and Processing

Sampling was conducted on days 30 and 60, with five sea cucumbers randomly selected from each replicate tank. Prior to sampling, feeding was suspended for 72 hours to empty the intestinal tract. After gently blotting surface moisture with filter paper, individuals were weighed and placed on ice. Coelomic fluid was carefully extracted using a 1 mL syringe inserted through the anus, immediately centrifuged at 4 ℃ and 4,000 r/min for 10 minutes, and the supernatant was aliquoted into 0.5 mL tubes and stored at -20 ℃ for subsequent immune assays. Following coelomic fluid collection, 0.35 mol/L potassium chloride solution was injected to induce evisceration, and the intestines were harvested. Respiratory trees attached to the posterior intestine were removed, and intestines were rinsed with ice-cold phosphate buffer (pH 7.0), blotted dry, weighed, and stored at -80 ℃ for digestive enzyme analysis.

Intestinal crude enzyme extracts were prepared by homogenizing the tissue in a glass grinder on ice for 10–15 minutes with phosphate buffer (pH 7.0) added at a ratio of 10 mL per gram of sample. The homogenate was centrifuged at 4 ℃ and 5,000 r/min for 30 minutes, and the resulting supernatant was stored at 4 ℃ and analyzed within 24 hours.

1.4 Measurement Indices

Growth performance indices were calculated as follows:
Survival rate (SR, %) = 100 × (final number of sea cucumbers / initial number of sea cucumbers)
Specific growth rate (SGR, %/day) = 100 × (ln final body weight -ln initial body weight) / feeding days
Gutted body weight rate (GBWR, %) = 100 × (fresh body wall weight / fresh whole body weight)

Apparent digestibility of dry matter (ADDM) was calculated as:
ADDM (%) = 100 -100 × (acid-insoluble ash content in feed / acid-insoluble ash content in feces) [9].

Intestinal protease activity was determined using the Folin-phenol method, and amylase activity was measured by the starch-iodine colorimetric method following procedures described in literature [10]. Protein concentration in crude enzyme extracts was quantified using a Coomassie brilliant blue assay kit (Nanjing Jiancheng Bioengineering Institute). Superoxide dismutase (SOD) and alkaline phosphatase (AKP) activities in coelomic fluid were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute) according to manufacturer protocols.

1.5 Statistical Analysis

All data are expressed as mean ± standard error. Statistical analysis was performed using SPSS 17.0 software with one-way ANOVA followed by Duncan' s multiple comparison test. Differences were considered significant at $P<0.05$.

2.1 Effects of Dietary XOS on Growth Performance and ADDM

As shown in Table 2 , dietary XOS supplementation had no significant effect on SR ($P>0.05$) but significantly improved SGR ($P<0.05$). The highest SGR (1.17%/day) was observed in the 0.045% XOS group, with SGR gradually decreasing at higher supplementation levels but remaining above the control group. The regression relationship between XOS supplemental level (X) and SGR (Y) was $Y=-151.68X^2+13.302X+0.8607$ ($R^2=0.9275$), indicating maximal SGR at 0.044% XOS supplementation (Fig. 1 [Figure 1: see original paper]). XOS supplementation influenced GBWR, though differences among groups were not significant ($P>0.05$). GBWR was slightly reduced at low XOS levels (0.015% and 0.030%) but increased above control values at supplementation levels 0.045%. ADDM was enhanced by XOS supplementation, with only the 0.030% group showing significantly higher values than the control (28.39% vs. 25.91%, $P<0.05$), while no significant differences were detected among supplemented groups ($P>0.05$).

2.2 Effects of Dietary XOS on Intestinal Digestive Enzyme Activities

Intestinal protease activity increased with feeding duration in all groups, whereas amylase activity increased over time only in the 0.015%, 0.030%, and 0.045% groups, decreasing in other groups (Table 3). On day 30, 0.015% XOS supplementation had minimal effect on protease activity ($P>0.05$), while 0.030%-0.060% XOS significantly elevated protease activity ($P<0.05$). Amylase activity was significantly reduced by 0.015%-0.045% XOS on day 30 ($P<0.05$) without a clear pattern, with only the 0.075% group showing significantly increased amylase activity ($P<0.05$). By day 60, both protease and amylase activities were substantially higher in all supplemented groups compared to the

control, with 0.015%–0.060% XOS producing significant increases ($P<0.05$). Peak activities were observed in the 0.045% group, with protease and amylase reaching 66.83 and 168.78 U/mg prot, respectively, representing 18.6% and 32.6% increases over the control ($P<0.05$).

2.3 Effects of Dietary XOS on SOD and AKP Activities in Coelomic Fluid

SOD and AKP activities in coelomic fluid generally increased over the experimental period in all supplemented groups except the 0.075% group, with higher XOS levels producing greater SOD elevation but no consistent pattern for AKP (Table 4). On day 30, 0.060%–0.075% XOS significantly increased SOD activity ($P<0.05$), while 0.030%–0.075% XOS significantly enhanced AKP activity ($P<0.05$), with maximal activities observed in the 0.075% group (22.64% and 186.08% higher than control for SOD and AKP, respectively, $P<0.05$). By day 60, no correlation existed between XOS level and enzyme activities, though 0.030%–0.075% XOS still significantly elevated SOD activity ($P<0.05$), peaking in the 0.060% group (72.45 U/mL). XOS supplementation increased AKP activity, but significant differences from the control were observed only in the 0.045% group at day 60 ($P<0.05$).

3.1 Effects of Dietary XOS on Growth Performance

XOS represents a novel aquafeed additive that selectively promotes beneficial bacteria such as *Bifidobacterium*, thereby enhancing animal growth and immunity. Chen et al. [7] reported that dietary XOS at 200 mg/kg significantly improved weight gain and SGR in *Litopenaeus vannamei*. Qiang et al. [11] found that 0.03% XOS significantly enhanced growth performance in juvenile hybrid tilapia (*Oreochromis niloticus* × *O. aureus*), with 0.03% being the optimal level. In the present study, 0.030%–0.075% XOS significantly improved growth of juvenile sea cucumbers, with an optimal level of 0.044%, slightly higher than previous reports. This discrepancy may be attributed to the slow movement and weak active feeding ability of sea cucumbers, combined with the powdered feed form, potentially causing partial XOS loss. Liang [8] reported that 0.06%–0.12% XOS significantly improved sea cucumber growth rate but did not determine an optimal level, which differs substantially from our findings. These variations may stem from differences in experimental conditions, including sea cucumber size (~2.14 g in Liang' s study), culture systems (indoor recirculating 40 L tanks), basal diet formulations, and unspecified XOS sources.

Digestibility is a crucial indicator of feed efficacy. Yingst [12] reported that deposit-feeding holothurians exhibit extremely low cellulase activity, resulting in low ADDM for *Zostera* powder-based diets. Sun et al. [13] and Seo et al. [14] demonstrated that sea cucumbers exhibit ADDM values of 36.0%–63.9% for formulated feeds composed primarily of digestible ingredients such as algal powder, soybean meal, and wheat flour. Xia [15] found that when sea cucumbers were fed diets containing 30% various algal powders and 70% sea mud, ADDM ranged

only from 9.63% to 15.84%, attributed to high proportions of sea mud and high-cellulose algal powders. In this study, ADDM values of 25.90%–28.40% fell between these ranges, likely because sea mud comprised 50% of the diet while the formulated portion contained readily digestible ingredients such as soybean meal and wheat middlings.

In summary, maximal SGR occurred at 0.044% XOS supplementation, while peak ADDM was observed at 0.030% XOS. This divergence may be related to enhanced growth at 0.030% XOS without concurrent effects on body wall composition, potentially explaining the initial decrease followed by increase in GBWR.

3.2 Effects of Dietary XOS on Intestinal Digestive Enzyme Activities

Oligosaccharides, including XOS, are generally indigestible by animals but can be metabolized by intestinal probiotics (e.g., *Bifidobacterium*, *Lactobacillus*), which stimulate intestinal epithelial cell proliferation and enhance digestive enzyme activities [16]. Chen et al. [6] reported that dietary XOS at 1,400 mg/kg significantly improved hepatopancreatic digestive enzyme activities in *L. vannamei*. Qiang et al. [11] observed that protease activities in tilapia hepatopancreas and intestine increased initially then decreased with increasing XOS levels. No previous studies have examined XOS effects on sea cucumber intestinal digestive enzymes. Our study evaluated both XOS dosage and feeding duration effects on protease and amylase activities. At 30 days, amylase activity decreased then increased with XOS level, while both protease and amylase activities exhibited an increase-then-decrease pattern at 60 days, with this trend becoming more pronounced over time, consistent with Qiang et al. [11]. This may be because beneficial bacteria produce volatile fatty acids during XOS fermentation, lowering intestinal pH and stimulating peristalsis [17]. Excessive XOS may lead to increased microbial metabolites, enhanced intestinal motility, reduced feed retention time, and excessive enzyme excretion or inhibition, thereby decreasing digestive enzyme activities. While XOS affects protease activity within a relatively short period, its influence on amylase requires longer exposure, though the underlying mechanisms warrant further investigation.

3.3 Effects of Dietary XOS on Immunity

Sea cucumber defense mechanisms rely on cellular and humoral immunity, supplemented by antioxidant enzymes that enhance immunity and maintain immune balance. SOD is a key enzyme in the antioxidant system and a primary indicator of organism health. When foreign substances invade, coelomocytes produce various hydrolases to digest and degrade foreign materials. AKP is a phosphomonoester hydrolase involved in detoxification systems and nutrient digestion. As a functional oligosaccharide, XOS acts as an adjuvant and immune modulator, binding to toxin or viral cell surfaces to slow antigen absorption and increase antigen efficacy [18]. XOS also promotes proliferation of beneficial bacteria such as *Bifidobacterium*, which can elevate antibody levels and acti-

vate macrophage phagocytic activity, thereby enhancing immune function [19]. Studies have shown that XOS can increase erythrocyte count and hemoglobin concentration while decreasing serum cholesterol and urea nitrogen in channel catfish (*Ictalurus punctatus*), effectively improving oxygen transport capacity and immunity [20]. Chen et al. [7] reported that serum immune factor activities in *L. vannamei* increased then decreased with feeding time and XOS level. Similar trends were observed in our study, where 0.030%–0.075% XOS significantly elevated SOD and AKP activities in coelomic fluid within 30 days, with activities continuing to increase through 60 days. As non-specific immune-related enzymes in coelomic fluid, SOD and AKP were enhanced by appropriate XOS levels through adjuvant and immunomodulatory effects, promoting beneficial bacterial proliferation and nutrient metabolism, thereby increasing synthesis of immune factors. However, excessive XOS may cause over-proliferation of certain bacteria or remain unutilized by beneficial microbes, potentially inhibiting *Bifidobacterium* activity and causing nutrient metabolism disorders [11,21], ultimately reducing immunity. Further studies are needed to elucidate the mechanisms of XOS dosage and application duration effects on immune parameters and to determine whether long-term feeding induces immune fatigue.

Conclusions

Dietary XOS supplementation can enhance intestinal digestive enzyme activities and immunity, thereby promoting growth of juvenile sea cucumbers.

The optimal dietary XOS supplementation range is 0.030%–0.060%, with 0.044% being the most effective level. A feeding duration of approximately two months is recommended.

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