Effects of Dietary Xylo-oligosaccharide Supplementation on Digestive Enzyme Activity, Intestinal Morphology, and Bacterial Count in Juvenile Litopenaeus vannamei (Postprint)

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

Abstract

This study aimed to investigate the effects of dietary xylo-oligosaccharides (XOS) on digestive enzyme activities, intestinal morphology, and bacterial counts in Pacific white shrimp (Litopenaeus vannamei). Eight hundred juvenile Pacific white shrimp with an average body weight of (0.67±0.02) g were randomly assigned to 4 groups, with 4 replicates per group and 50 shrimp per replicate. The control group was fed a basal diet, while the experimental groups were fed test diets supplemented with 200, 400, and 600 mg/kg XOS. The feeding trial lasted for 42 days. The results showed: 1) Compared with the control group, the intestinal amylase activity and hepatopancreatic protease activity of shrimp in the experimental groups were significantly increased ($P<0.05$); the intestinal and hepatopancreatic protease activities in the 200 mg/kg group, the intestinal and hepatopancreatic lipase activities in the 400 mg/kg group, and the gastric protease activity in the 400 mg/kg group were significantly elevated ($P<0.05$). 2) Compared with the control group, the hepatopancreatic total protein content, intestinal wall thickness, and intestinal villus height in the 400 mg/kg group were all significantly increased ($P<0.05$). 3) Compared with the control group, the total intestinal bacteria and Bifidobacterium counts of shrimp in the experimental groups were significantly increased ($P<0.05$), while the intestinal Vibrio count was significantly decreased ($P<0.05$). In conclusion, dietary XOS supplementation can enhance digestive enzyme activities, improve intestinal morphology, increase total intestinal bacteria and Bifidobacterium counts, and reduce Vibrio counts in juvenile Pacific white shrimp.

Full Text

Effects of Dietary Xylo-Oligosaccharides on Digestive Enzyme Activities, Intestinal Morphology and Bacterial Numbers of Juvenile Litopenaeus vannamei

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Abstract

This experiment was conducted to investigate the effects of dietary xylo-oligosaccharides (XOS) on digestive enzyme activities, intestinal morphology, and bacterial numbers of juvenile Litopenaeus vannamei. Eight hundred juvenile L. vannamei with an average body weight of (0.67±0.02) g were randomly assigned to four groups with four replicates per group and 50 shrimp per replicate. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 200, 400, and 600 mg/kg XOS, respectively. The feeding trial lasted for 42 days. The results showed: (1) Compared with the control group, intestinal amylase activity and hepatopancreas protease activity were significantly increased in the experimental groups ($P<0.05$). Specifically, intestinal and hepatopancreas protease activities in the 200 mg/kg group, intestinal and hepatopancreas lipase activities in the 400 mg/kg group, and stomach protease activity in the 400 mg/kg group were significantly elevated ($P<0.05$). (2) The hepatopancreas total protein content, intestinal wall thickness, and intestinal villus height in the 400 mg/kg group were significantly higher than those in the control group ($P<0.05$). (3) The intestinal total bacteria and Bifidobacterium numbers in the experimental groups were significantly higher ($P<0.05$), while the intestinal Vibrio number was significantly lower ($P<0.05$) compared with the control group. In conclusion, dietary XOS supplementation can improve digestive enzyme activities and intestinal morphology, increase intestinal total bacteria and Bifidobacteria numbers, and decrease Vibrio numbers in juvenile L. vannamei.

Keywords: xylo-oligosaccharides; Litopenaeus vannamei; digestive enzyme activities; intestinal morphology; intestinal bacteria

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Introduction

Litopenaeus vannamei is one of the three major shrimp species with the highest aquaculture production worldwide and represents the dominant cultured shrimp species and an important export aquatic product in China. It offers significant economic benefits due to its rapid growth and strong disease resistance. However, with the intensification and scaling-up of farming practices and the increasing replacement of fish meal in feed formulations, shrimp diseases have become more prevalent. The misuse of antibiotics and other drugs has led to metabolic disorders, decreased immunity and disease resistance, and compromised intestinal health, seriously threatening the sustainable development of the shrimp industry. Currently, regulating intestinal health through nutritional approaches to reduce disease incidence and drug usage has become a major focus of modern nutrition research. Therefore, identifying and developing green, safe, and effective antibiotic-free additives to regulate shrimp intestinal health is of great significance [1].

Functional oligosaccharides represent a novel class of feed additives [2]. Xylo-oligosaccharides (XOS) are a common type of functional oligosaccharide, consisting of low-degree polymerized sugars with xylobiose and xylotriose as the main active components. Compared with other functional oligosaccharides, XOS possesses unique properties, including resistance to degradation by animal digestive enzymes, exceptional acid and thermal stability, no compatibility contraindications, significant proliferation of Bifidobacterium, and pronounced effects even at low supplementation levels. Previous studies have demonstrated that XOS can maintain intestinal microbial stability, promote beneficial bacteria proliferation, inhibit harmful bacteria growth, improve digestive tract structure, enhance immunity and antioxidant capacity, and promote growth in livestock and poultry [3-6]. Research has also shown that dietary XOS or synbiotics can increase intestinal protease and amylase activities in sea cucumber (Apostichopus japonicus Selenka) and grass carp (Ctenopharyngodon idellus) [7-8], improve hepatopancreas trypsin and lipase activities in grass carp [8], and increase beneficial bacteria such as Bifidobacterium while reducing harmful bacteria like Escherichia coli or Vibrio in grass carp [9], Nile tilapia (Oreochromis aureus×O. niloticus) [10], and L. vannamei [11]. However, no studies have reported the effects of XOS on intestinal digestive enzyme activities and intestinal function in L. vannamei. Therefore, based on our previous research on the effects of XOS on growth performance, immunity, and antioxidant function in juvenile L. vannamei [12], this experiment investigated the effects of dietary XOS on digestive enzyme activities, intestinal morphology, and bacterial numbers to provide a theoretical basis for XOS application in crustacean feeds.

Materials and Methods

1.1 Experimental Diets

A basal diet was formulated using fish meal, soybean meal, and rapeseed meal as the main protein sources; fish oil and soybean lecithin as the main lipid sources; and wheat flour as the main carbohydrate source. The basal diet contained 41.90% crude protein and 6.03% crude lipid, with its composition and nutrient levels shown in Table 1 . Three experimental diets were prepared by supplementing the basal diet with 200, 400, and 600 mg/kg XOS (purchased from Shandong Longlive Bio-Technology Co., Ltd., purity 95%). All ingredients were ground to pass through a 60-mesh sieve and processed into 1.2–1.5 mm diameter pellets using an SLX-80 twin-screw extruder. The pellets were dried at 55°C, cooled, sieved, and stored in sealed bags at -20°C until use.

1.2 Experimental Design and Management

Juvenile L. vannamei shrimp were obtained from Charoen Pokphand Group (Zhuhai Hatchery). After a 4-week acclimation period with a temporary feed, the feeding trial was conducted in an indoor recirculating aquaculture system at the Aquaculture Research Laboratory of the Institute of Animal Science, Guangdong Academy of Agricultural Sciences. Shrimp were cultured in 16 fiberglass tanks (volume 350 L, diameter 80 cm, height 70 cm, water volume 300 L) with a water inflow rate of 3.4 L/min. Eight hundred healthy juvenile shrimp with an individual weight of (0.67±0.02) g were randomly divided into four groups with four replicates per group and 50 shrimp per replicate. Each group was fed either the basal diet or one of the three experimental diets, designated as G0 (control), G200, G400, and G600 groups. Shrimp were fed to satiation three times daily at 08:00, 14:00, and 20:00, with residual feed collected after 30 minutes. Shrimp health status was observed daily, and mortality was recorded. The water source was brackish water mixed from seawater and tap water, filtered and disinfected before use. Water salinity was maintained at 4.0‰–5.0‰ under natural light conditions. Water temperature was 28.5–32.0°C, pH 7.8–8.0, dissolved oxygen >6.0 mg/L, ammonia nitrogen 0.1 mg/L, and nitrite 0.01 mg/L. The experimental period lasted 42 days.

1.3 Sample Collection and Analysis

1.3.1 Sample Collection At the end of the feeding trial, shrimp were fasted for 12 hours. Three shrimp from each replicate were randomly selected, and their intestines were dissected and fixed in 10% formalin solution for intestinal morphology analysis. Another three shrimp per replicate were dissected on ice plates to isolate the stomach, intestine, and hepatopancreas. The stomach and intestine were opened and rinsed with shrimp physiological saline to remove contents, blotted dry with filter paper, placed in Eppendorf tubes, and stored at -20°C for digestive enzyme activity analysis. Additionally, three shrimp per replicate were aseptically dissected to obtain intestines, which were placed in

Eppendorf tubes and stored at 4°C for bacterial enumeration.

For tissue homogenate preparation, hepatopancreas, stomach, and intestine samples were thawed at 4°C. A portion of each sample was weighed, minced, and placed in a glass homogenizer with six volumes of ice-cold physiological saline. Homogenization was performed on ice using a T10 basic electric glass homogenizer (IKA). After thorough homogenization, the mixture was transferred to a centrifuge tube, and three additional volumes of ice-cold saline were used to rinse the homogenizer and combined to make a 10% tissue homogenate. A portion was stored at 4°C for lipase activity measurement, while the remainder was centrifuged at 3,500 r/min for 10 minutes. The supernatant was divided into two portions: one stored at 4°C for protease and amylase activity measurement, and the other at -80°C for total protein (TP) content determination. Intestinal samples stored at 4°C were similarly processed into 10% homogenates.

1.3.2 Digestive Enzyme Activity Assay Protease, amylase, and lipase activities were measured according to the methods of Wang et al. [13], the 3,5-dinitrosalicylic acid (DNS) colorimetric method [14], and the polyvinyl alcohol olive oil emulsion hydrolysis method [15], respectively.

1.3.3 TP Content Determination in Homogenate Supernatant Hepatopancreas and intestinal homogenate supernatants were prepared, and TP content was determined using the Coomassie brilliant blue method with bovine serum albumin as the standard [16].

1.3.4 Intestinal Tissue Section Observation Intestines fixed in 10% formalin were dehydrated using conventional methods, embedded in paraffin, sectioned serially, stained with hematoxylin-eosin (HE), and mounted with balsam for morphological and structural observation under an optical microscope. Measurements were selected following the random equidistance principle in histological sample collection and image data measurement. Intestinal wall thickness was measured as the average of 30 random measurements of the muscularis layer thickness per section. Intestinal villus height was measured as the vertical distance from the villus tip to the crypt base, with the average of 10 random measurements per section.

1.3.5 Intestinal Bacterial Count Determination Bacterial counts were determined using the dilution plating method described by Li [17]. The 10% homogenate was serially diluted 10-fold, thoroughly mixed, and 0.1 mL aliquots of 10^{3}, 10 , and 10 dilutions were plated in triplicate for each replicate. Plates were incubated at 28°C in darkness at pH 7.0–7.4. Total bacteria and Vibrio were cultured for 24 hours, while Lactobacillus was cultured for 48 hours. As an anaerobe, Bifidobacterium was cultured by pouring a layer of 40°C Bifidobacterium agar (BBL agar) after plating and incubating for 48 hours under the same conditions. Microbial colony counts were performed based on the principle of

countable colonies. The dilution yielding 30–300 colonies was selected, and the average of three parallel plates was calculated and expressed as log CFU/g of intestinal content. All culture media were purchased from Guangdong Huankai Microbial Sci. & Tech. Co., Ltd.

1.4 Statistical Analysis Data were expressed as means ± standard deviation. Statistical analysis was performed using SPSS 17.0 software. One-way ANOVA was used for data analysis, and Duncan' s multiple comparison test was applied when significant differences were detected among groups. Statistical significance was set at $P<0.05$.

Results

2.1 Effects of Dietary XOS on Digestive Enzyme Activities in Intestine, Hepatopancreas, and Stomach of L. vannamei

As shown in Table 2 , dietary XOS supplementation significantly increased intestinal amylase activity and hepatopancreas protease activity compared with the control group ($P<0.05$). Other digestive enzyme activities in the intestine, hepatopancreas, and stomach were also improved to varying degrees. The G200 group exhibited the highest intestinal, hepatopancreas, and stomach amylase activities, which were significantly higher than those of the control group ($P<0.05$). The G200 group also showed the highest intestinal protease activity, followed by the G400 group, with both being significantly higher than the control group ($P<0.05$). The G400 group had the highest hepatopancreas and stomach protease activities, which were significantly higher than the control group ($P<0.05$). The G400 group showed the highest intestinal lipase activity, significantly higher than the control group ($P<0.05$). Hepatopancreas lipase activity increased with increasing dietary XOS levels, with the G400 and G600 groups being significantly higher than the control group ($P<0.05$). No significant differences were observed in stomach lipase activity among all groups ($P>0.05$).

2.2 Effects of Dietary XOS on TP Content in Hepatopancreas and Intestine of L. vannamei

As shown in Table 3 , TP content in both hepatopancreas and intestine was higher in all experimental groups than in the control group, showing a trend of initial increase followed by decrease. The G400 group had the highest hepatopancreas TP content, which was significantly higher than the control group ($P<0.05$) but not significantly different from other experimental groups ($P>0.05$). The G400 group also showed the highest intestinal TP content, although no significant differences were observed among groups ($P>0.05$).

2.3 Effects of Dietary XOS on Intestinal Wall Thickness and Villus Height of L. vannamei

As shown in Table 4 , both intestinal wall thickness and villus height increased initially and then decreased with increasing dietary XOS levels, with the G400 group showing the highest values that were significantly higher than the control group ($P<0.05$). The intestinal wall thickness of the G400 group was not significantly different from other experimental groups ($P>0.05$). Intestinal tissue sections of L. vannamei from each group are shown in Figure 1 [Figure 1: see original paper].

2.4 Effects of Dietary XOS on Intestinal Bacterial Numbers of L. vannamei

As shown in Table 5 , the intestinal total bacteria and Bifidobacterium numbers in all experimental groups were significantly higher than those in the control group ($P<0.05$), while the intestinal Vibrio number was significantly lower ($P<0.05$). Lactobacillus numbers in the experimental groups were higher than in the control group to varying degrees, but no significant differences were observed among groups ($P>0.05$).

Discussion

3.1 Effects of Dietary XOS on Digestive Enzyme Activities of L. vannamei

The intestine is the primary site for digestion and absorption in animals, and its digestive enzyme activities reflect fundamental digestive physiological characteristics that directly affect nutrient absorption and utilization, thereby influencing growth and development. These activities serve as important indicators of digestive capacity. In this study, intestinal amylase, protease, and lipase activities increased initially and then decreased with increasing dietary XOS levels, showing a similar trend to the weight gain rate and specific growth rate observed in our previous study [12]. The G200 group exhibited significantly higher intestinal protease and amylase activities and the best growth performance [12], indirectly indicating a correlation between intestinal digestive enzyme activities and growth indicators. These results are consistent with findings on digestive enzyme activities in the intestine of gibel carp (Carassius auratus gibelio) [18], Nile tilapia [19], sea cucumber [7], and L. vannamei [11]. Sugita et al. [20] reported that intestinal microflora plays a crucial role in starch digestion in freshwater fish. Beneficial bacteria such as Bifidobacterium and Lactobacillus and their metabolites can promote digestive enzyme secretion and intestinal peristalsis, thereby enhancing digestive enzyme activities, whereas harmful bacteria can damage intestinal villi and microvilli, reducing enzyme secretion [21]. This study confirmed that XOS promotes the proliferation of beneficial bacteria (Bifidobacterium, Lactobacillus) and inhibits harmful Vibrio growth. Additionally, XOS increased intestinal villus height and wall thickness. Therefore, XOS

appears to promote digestive enzyme secretion primarily by improving the microbial community and intestinal tissue structure. However, the inconsistent trend between intestinal TP content and intestinal protease activity suggests that protein deposition in the intestine is influenced not only by protease activity but also by other factors such as intestinal microbial numbers.

Few studies have investigated the effects of oligosaccharides on digestive enzyme activities in hepatopancreas and stomach, with inconsistent results. Dietary fructooligosaccharide (FOS) showed no significant effects on digestive enzyme activities in broilers [22] or L. vannamei [23]. In contrast, Xiao et al. [24] and Xiong et al. [25] found that appropriate levels of oligosaccharides significantly increased hepatopancreas protease activity in Chinese soft-shelled turtle (Trionyx sinensis) and gibel carp, respectively. In this study, XOS significantly improved digestive enzyme activities in hepatopancreas and stomach, though these activities did not correlate with growth performance as shown in our previous study. However, the trend in hepatopancreas TP content was consistent with that of hepatopancreas protease activity, indicating a relationship between protein deposition and protease activity in the hepatopancreas.

3.2 Effects of Dietary XOS on Intestinal Morphology of L. vannamei

Intestinal health is essential for rapid growth and disease resistance in animals. Intestinal mucosal morphology provides the structural basis for digestion and absorption, and increased intestinal wall thickness, crypt depth, and villus height can enhance digestive and absorptive capacity. Although progress has been made in studying oligosaccharide effects on intestinal morphology, limited research exists on XOS. Previous studies have shown that dietary oligosaccharides can significantly improve intestinal morphology in tilapia [10,26-27], grass carp [28], and Schizothorax prenanti [29]. This study demonstrated that dietary XOS increased intestinal wall thickness and villus height in L. vannamei, which would increase absorptive surface area and improve nutrient utilization efficiency [30]. This effect likely occurs because XOS serves as a proliferation factor for beneficial bacteria, promoting their growth, regulating microecological balance, and improving intestinal tissue structure and metabolic function [31]. Additionally, short-chain fatty acids (butyrate, propionate, and acetate) produced by beneficial bacteria can serve as energy sources to promote mucosal cell proliferation, contributing to increased intestinal wall thickness and villus height [32-33].

3.3 Effects of Dietary XOS on Intestinal Bacterial Numbers of L. vannamei

Intestinal bacterial composition and numbers are important indicators of intestinal health, significantly influencing intestinal cell proliferation, tissue morphology, nutrient digestion and absorption, immunity, and normal growth. The effects of oligosaccharides on intestinal bacteria have been reported in livestock, poultry, and aquatic animals. Dietary XOS can increase Bifidobacterium and Lactobacillus numbers while decreasing harmful Escherichia coli in livestock and

poultry [34-38]. Similar effects have been observed in aquatic animals such as Nile tilapia [10], grass carp [8], and sea cucumber [39]. This study confirmed that dietary XOS had no significant effect on intestinal Lactobacillus numbers in L. vannamei but significantly increased Bifidobacterium and total bacteria numbers while decreasing Vibrio numbers.

XOS serves as a carbon source for beneficial bacteria, promoting their proliferation and competitive advantage while inhibiting harmful bacteria colonization. Additionally, beneficial bacteria like Bifidobacterium can form a biological barrier preventing pathogen invasion [39-40]. Pestova et al. [41] found that XOS contains structural substances similar to lectins on many pathogenic microorganisms, allowing competitive binding to pathogens. Since XOS is not degraded by endogenous digestive enzymes, it can carry bound pathogens out of the intestine, preventing their proliferation. Through these mechanisms, XOS significantly promotes beneficial bacteria and inhibits harmful bacteria.

Conclusion

Dietary supplementation with appropriate levels of XOS can significantly improve digestive enzyme activities, enhance intestinal morphology, increase intestinal total bacteria and Bifidobacteria numbers, and decrease Vibrio numbers in juvenile Litopenaeus vannamei.

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