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

Authors: Chen Xiaoying, Wang Guoxia, Sun Yuping, Chen Bing, Mo Wenyan, Zhao Hongxia, Cao Junming, Huang Yanhua

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

Abstract

This experiment 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 selected and randomly divided into 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 in the basal diet. The experimental period lasted 42 days. The results showed: 1) Compared with the control group, the intestinal amylase activity and hepatopancreatic protease activity in the experimental groups were significantly increased ($P<0.05$), and 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 significantly increased ($P<0.05$). 3) Compared with the control group, the total intestinal bacteria and Bifidobacterium counts 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

CHEN Xiaoying[1,2,3], , WANG Guoxia[1,2,3], , SUN Yuping[1,2,3], , CHEN Bing[1,2,3], , MO Wenyan[1,2,3], , ZHAO Hongxia[1,2,3], , CAO Junming[1,2,3], *HUANG Yanhua[1,2,3],*

[1]Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China
[2]Key Laboratory of Animal Nutrition and Feed Science in South China, Ministry of Agriculture, Guangzhou 510640, China
[3]Guangdong Key Laboratory of Animal Breeding and Nutrition, Guangzhou 510640, China
Guangzhou Fishtech Biotechnology Co., Ltd., Guangzhou 510640, China

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*. A total of 800 juvenile shrimp 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 than that in the control group ($P<0.05$). In conclusion, dietary XOS supplementation can improve digestive enzyme activities and intestinal morphology, increase intestinal total bacteria and *Bifidobacterium* numbers, and decrease *Vibrio* number in juvenile *L. vannamei*.

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

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 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, using nutritional strategies to regulate intestinal health, reduce disease incidence, and minimize 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), a common type of functional oligosaccharide, are low-degree polymerized sugars whose main active components are xylobiose and xylotriose. Compared with other functional oligosaccharides, XOS possesses unique properties, including resistance to degradation by animal digestive enzymes, distinctive acid and thermal stability, no compatibility contraindications, and the ability to significantly proliferate *Bifidobacterium* at low supplementation levels. Studies have demonstrated that XOS can maintain the stability of intestinal microflora, promote the proliferation of beneficial bacteria, inhibit the growth of harmful bacteria, 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], enhance 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 gut 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 study investigated the impacts 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 Biotechnology 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* were obtained from Charoen Pokphand Group' s Zhuhai Hatchery. After a 4-week acclimation period with a commercial diet, 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 (350 L capacity, 80 cm diameter, 70 cm height, 300 L water volume) with a water inflow rate of 3.4 L/min. Eight hundred healthy shrimp with an individual weight of (0.67±0.02) g were randomly allocated into four groups with four replicates each (50 shrimp per replicate). The groups were fed the basal diet (control, G0) or the three experimental diets (G200, G400, and G600). Shrimp were hand-fed to apparent satiation three times daily at 08:00, 14:00, and 20:00, with uneaten feed collected 30 minutes after feeding. Shrimp health status and mortality were monitored daily. The water source was brackish water mixed from seawater and tap water, filtered and disinfected before use. Water salinity was 4.0–5.0‰. The experiment was conducted under natural light with water temperature maintained at 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 trial 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 per 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 placed on ice, and their stomachs, intestines, and hepatopancreases were dissected. The stomachs and intestines were opened and rinsed with shrimp physiological saline, blotted dry with filter paper, placed in Eppendorf tubes, and stored at -20°C for digestive enzyme activity analysis. Simultaneously, three shrimp per replicate were randomly selected, and their intestines were aseptically dissected and placed in Eppendorf tubes at 4°C for bacterial enumeration.

For tissue homogenate preparation, hepatopancreas, stomach, and intestine sam-

ples stored at -20°C were thawed at 4°C. A portion of each sample was weighed, minced, and placed in a glass homogenizer with 6 volumes of ice-cold physiological saline. The mixture was homogenized on ice using a T10 basic electric glass homogenizer (IKA). After thorough homogenization, the suspension was transferred to a centrifuge tube, and the homogenizer was rinsed with 3 volumes of ice-cold physiological saline, which was added to the same tube to prepare a 10% tissue homogenate. A portion was stored at 4°C for lipase activity determination, while the remainder was centrifuged at 3,500 r/min for 10 minutes. The supernatant was divided into two aliquots: one stored at 4°C for protease and amylase activity determination, and the other at -80°C for total protein (TP) content measurement. Intestinal samples stored at 4°C were processed similarly to prepare 10% intestinal homogenates.

1.3.2 Digestive Enzyme Activity Assay

Protease, amylase, and lipase activities were determined 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 Supernatants

Hepatopancreas and intestinal homogenate supernatants were prepared, and TP content was measured using the Coomassie brilliant blue method with bovine serum albumin as the standard [16].

1.3.4 Intestinal Histological Observation

Intestinal samples fixed in 10% formalin were dehydrated, paraffin-embedded, sectioned serially, stained with hematoxylin-eosin (HE), and mounted. Morphological and structural changes were observed under an optical microscope. Measurements were taken following the random systematic sampling principle for histological specimens. Intestinal wall thickness was measured at 30 randomly selected muscular layer sites per slice and averaged. Intestinal villus height was measured as the vertical distance from the villus tip to the crypt base, with 10 randomly selected sites measured per slice and averaged.

1.3.5 Intestinal Bacterial Enumeration

The dilution plating method described by Li [17] was used. The 10% homogenate was serially diluted 10-fold, vortexed thoroughly, and 0.1 mL aliquots of 10^{-3}, 10^{-} , and 10^{-} dilutions were plated in triplicate per 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 lactic acid bacteria were cultured for 48 hours. *Bifidobacterium*, being anaerobic, was cultured by pouring a 40°C layer of BBL agar medium after plating and incubating at 28°C in darkness for 48 hours. Based on colony count principles, plates with 30–300 colonies were selected, and the average of three replicates was calculated. Bacterial numbers per gram of intestine were expressed as log CFU/g. All culture media were purchased from Guangdong Huankai Microbial Co., Ltd.

1.4 Statistical Analysis Data are presented as mean ± standard deviation. Statistical analysis was performed using SPSS 17.0 software. One-way ANOVA was used to analyze the data, and Duncan' s multiple range test was applied for post-hoc comparisons when significant differences were detected. Differences were considered significant 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 enhanced to varying degrees. The 200 mg/kg group exhibited the highest intestinal amylase activity, significantly higher than the control ($P<0.05$). Intestinal protease activity was highest in the 200 mg/kg group, followed by the 400 mg/kg group, with both significantly exceeding the control ($P<0.05$). Hepatopancreas and stomach protease activities were highest in the 400 mg/kg group, significantly higher than the control ($P<0.05$). Intestinal lipase activity peaked in the 400 mg/kg group, significantly higher than the control ($P<0.05$). Hepatopancreas lipase activity increased with XOS supplementation, with the 400 and 600 mg/kg groups significantly higher than the control ($P<0.05$). 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* Table 3 shows that TP content in both hepatopancreas and intestine was higher in all experimental groups than in the control, following an initial increase then decrease trend. The 400 mg/kg group had the highest hepatopancreas TP content, significantly greater than the control ($P<0.05$) but not significantly different from other experimental groups ($P>0.05$). The 400 mg/kg group also showed the highest intestinal TP content, though no significant differences were detected among groups ($P>0.05$).

2.3 Effects of Dietary XOS on Intestinal Wall Thickness and Villus Height of *L. vannamei* As presented in Table 4 , both intestinal wall thickness and villus height increased initially then decreased with increasing XOS supplementation, peaking in the 400 mg/kg group, which was significantly higher than the control ($P<0.05$). The 400 mg/kg group' s intestinal wall thickness did not differ significantly from other experimental groups ($P>0.05$). Intestinal histological sections for 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* Table 5 demonstrates that intestinal total bacteria and *Bifidobacterium* numbers were significantly higher in all experimental groups compared with the control ($P<0.05$), while *Vibrio* numbers were significantly lower ($P<0.05$). *Lactobacillus* numbers were numerically higher in experimental groups than in the control, 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. Digestive enzyme activity reflects fundamental digestive physiological characteristics and directly influences nutrient absorption and utilization, thereby affecting growth and development. It serves as a crucial indicator of digestive capacity. In this study, intestinal amylase, protease, and lipase activities initially increased then decreased with increasing XOS supplementation, mirroring the trend observed in our previous study for weight gain rate and specific growth rate. The 200 mg/kg group showed significantly higher intestinal protease and amylase activities and the best growth performance [12]. These results indirectly suggest a correlation between intestinal digestive enzyme activities and growth parameters, consistent with findings in 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 vital 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 activity, whereas harmful bacteria can damage intestinal villi and microvilli, reducing enzyme secretion [21]. This study confirmed that XOS promoted the proliferation of beneficial bacteria (*Bifidobacterium*, *Lactobacillus*) while inhibiting harmful *Vibrio* growth. Additionally, XOS increased villus height and wall thickness. Therefore, XOS appears to enhance digestive enzyme secretion primarily by improving microbial balance and intestinal structure. However, the inconsistent trend between intestinal TP content and protease activity suggests that protein deposition may also be influenced by other factors, such as intestinal bacterial numbers.

Few studies have examined the effects of oligosaccharides on hepatopancreas and stomach digestive enzyme activities, with inconsistent results. Dietary fructooligosaccharide (FOS) supplementation showed no significant effect on digestive enzyme activities in broilers [22] or *L. vannamei* [23]. Conversely, Xiao et al. [24] and Xiong et al. [25] found that appropriate oligosaccharide supplementation significantly increased hepatopancreas protease activity in Chinese soft-shelled turtle (*Trionyx sinensis*) and gibel carp. In this study, XOS significantly enhanced hepatopancreas and stomach digestive enzyme activities,

though these did not correlate with growth performance as shown in our previous study. However, the trend in hepatopancreas TP content aligned with hepatopancreas protease activity, indicating a relationship between protein deposition and protease activity in this organ.

3.2 Effects of Dietary XOS on Intestinal Morphology of *L. vannamei* Intestinal health is essential for rapid growth and disease resistance. Intestinal mucosal morphology provides the structural basis for digestion and absorption, and increased wall thickness, crypt depth, and villus height can enhance digestive capacity. While research on oligosaccharides and intestinal morphology has progressed, studies on XOS remain limited. Previous work demonstrated that appropriate oligosaccharide supplementation improved intestinal morphology in tilapia [10,26-27], grass carp [28], and *Schizothorax prenanti* [29]. This study found that XOS increased intestinal wall thickness and villus height in shrimp, which would expand 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 structure and metabolic function [31]. Additionally, short-chain fatty acids (butyrate, propionate, acetate) produced by beneficial bacteria can serve as energy sources for mucosal cell proliferation, contributing to increased wall thickness and villus height [32-33].

3.3 Effects of Dietary XOS on Intestinal Bacterial Numbers of *L. vannamei* Intestinal bacterial composition and abundance are important indicators of gut health, profoundly 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 and aquatic animals. Dietary XOS supplementation increased *Bifidobacterium* and *Lactobacillus* while decreasing harmful *E. coli* in livestock [34-38], and similar effects were observed in Nile tilapia [10], grass carp [8], and sea cucumber [39]. This study confirmed that XOS significantly increased total bacteria and *Bifidobacterium* numbers while reducing *Vibrio* numbers in *L. vannamei*, though *Lactobacillus* numbers were not significantly affected. XOS serves as a carbon source for beneficial bacteria, promoting their proliferation and competitive advantage while inhibiting pathogen colonization. Additionally, beneficial bacteria like *Bifidobacterium* form a biological barrier against pathogen invasion [39-40]. Pestova et al. [41] found that XOS contains structural components similar to lectins on pathogenic microbes, allowing competitive binding. Since XOS resists degradation by digestive enzymes, it can bind to and remove pathogens from the intestine, preventing their proliferation. Through these mechanisms, XOS significantly promotes beneficial bacteria while inhibiting harmful bacteria.

Conclusion

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

References

[1] MITSUOKA T. Prebiotics and intestinal flora[J]. Bioscience & Microflora, 2010, 21(1): 3-12.
[2] FRANCIS R J, BROUNS F. Immune-stimulating and gut health-promoting properties of Short-chain Fructo-oligosaccharides[J]. Nutrition Reviews, 2002, 60(10): 326-334.
[3] DE MAESSCHALCK C, EECKHAUT V, MAERTENS L, et al. Effects of xylo-oligosaccharides on broiler chicken performance and microbiota[J]. Applied and Environmental Microbiology, 2015, 81(17): 5880-5888.
[4] 石宁. 地衣芽孢杆菌和低聚木糖及乳酸对肉鸡肠绒组织及肠道菌群的影响 [D]. 硕士学位论文. 郑州: 河南工业大学, 2010.
[5] 杨海英, 杨在宾, 杨维仁, 等. 益生素和低聚木糖对断奶仔猪生产性能、消化酶活性、血液指标和肠道微生物的影响 [J]. 中国兽医学报, 2009, 29(7): 914-919.
[6] 刘亚利, 刑贞召, 翟蒲荣. 低聚木糖对仔猪生产性能和肠道菌群的影响 [J]. 西北农业学报, 2009, 18(6): 63-65.
[7] 李君华, 刘佳亮, 曹学彬, 等. 饲料中添加低聚木糖对仿刺参幼参生长性能、肠道消化酶活力和免疫力的影响 [J]. 动物营养学报, 2016, 28(8): 2534-2541.
[8] 姚东林, 邹青, 刘文斌, 等. 地衣芽孢杆菌和低聚木糖对草鱼生长性能、肠道菌群和消化酶活性的影响 [J]. 大连海洋大学学报, 2014, 29(2): 136-140.
[9] 熊娟, 吴志新, 张朋, 等. 投喂低聚木糖对草鱼肠道菌群的影响 [J]. 微生物学通报, 2011, 38(3): 317-321.
[10] 张荣斌, 曹俊明, 黄燕华, 等. 低聚木糖对奥尼罗非鱼肠道形态、菌群组成和抗嗜水气单胞菌感染的影响 [J]. 上海海洋大学学报, 2012, 21(2): 233-240.
[11] 胡毅. 凡纳滨对虾饲料配方优化及几种饲料添加剂的应用 [D]. 硕士学位论文. 青岛: 中国海洋大学, 2007.
[12] 陈晓瑛, 曹俊明, 黄燕华, 等. 饲料中添加低聚木糖对凡纳滨对虾幼虾生长性能、非特异性免疫力、抗氧化功能及抗对虾白斑综合征病毒能力的影响 [J]. 动物营养学报, 2014, 26(8): 2397-2407.
[13] 刘玉梅, 朱谨钊. 对虾消化酶的研究 [J]. 海洋科学, 1984, 8(5): 144-159.
[14] 潘鲁青, 王克行. 中国对虾幼体消化酶活力的实验研究 [J]. 水产学报, 1997, 21(1): 26-31.
[15] 朱俭. 生物化学实验 [M]. 上海: 上海科学技术出版社, 1981: 190-193.
[16] BRADFORD M M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding[J]. Analytical Biochemistry, 1976, 72(1/2): 248-254.
[17] 李继秋. 对虾微生态制剂的研究和应用 [D]. 博士学位论文. 青岛: 中国海洋大学, 2004.
[18] 刘波, 谢骏, 刘文斌, 等. 地衣芽孢杆菌与低聚木糖对异育银鲫消化酶活性、肠道菌群及生长的

影响 [J]. 大连水产学院学报, 2006, 21(4): 336-340.
[19] 强俊, 王辉, 李瑞伟, 等. 低聚木糖对奥尼罗非鱼幼鱼生长、体成分和消化酶活力的影响 [J]. 淡水渔业, 2009, 39(6): 63-68.
[20] SUGITA H, SHIBUYA K, SHIMOOKA H, et al. Antibacterial abilities of intestinal bacteria in freshwater cultured fish[J]. Aquaculture, 1996, 145(1/2): 195-203.
[21] 高丽松. 消化生理与保健 [M]. 北京: 中国医药科技出版社, 1998: 173-230.
[22] 胡彩虹, 占秀安. 果寡糖对肉鸡消化机能的影响 [J]. 中国粮油学报, 2003, 18(1): 49-54.
[23] 黄燕华, 王国霞, 刘襄河, 等. 低聚木糖对凡纳滨对虾生长及消化道主要消化酶活性的影响 [J]. 华南农业大学学报, 2010, 31(3): 61-64.
[24] 肖明松, 王志耕, 崔峰, 等. 果寡糖和糖萜素对中华鳖生长性能及免疫功能的影响 [J]. 水利渔业, 2004, 24(3): 20-22.
[25] 熊沈学, 刘文斌, 方星星. 低聚木糖梯度添加对异育银鲫生长及肠道消化酶活性的影响 [J]. 畜牧与兽医, 2005, 37(10): 23-24.
[26] 刘爱君, 冷向军, 李小勤, 等. 甘露寡糖对奥尼罗非鱼 (*Oreochromis niloticus*×*O. aureus*) 生长、肠道结构和非特异性免疫的影响 [J]. 浙江大学学报 (农业与生命科学版), 2009, 35(3): 329-336.
[27] 田娟, 孙立威, 文华, 等. 壳寡糖对吉富罗非鱼幼鱼生长性能、前肠组织结构及肠道主要菌群的影响 [J]. 中国水产科学, 2013, 20(3): 561-568.
[28] 徐介民. 壳聚糖对草鱼生长影响的研究 [D]. 硕士学位论文. 长沙: 湖南农业大学, 2008.
[29] 曾丽萍. 两种多糖对齐口裂腹鱼肠道消化酶、形态及菌群的影响 [D]. 硕士学位论文. 雅安: 四川农业大学, 2015.
[30] CASPARY F W. Physiology and pathophysiology of intestinal absorption[J]. The American Journal of Clinical Nutrition, 1992, 55(1S): 299S-308S.
[31] MOURÃO J L, PINHEIRO V, ALVES A, et al. Effect of mannan oligosaccharides on the performance, intestinal morphology and cecal fermentation of fattening rabbits[J]. Animal Feed Science and Technology, 2006, 126(1/2): 107-120.
[32] DUAN Y F, ZHANG Y, DONG H B, et al. Effect of dietary *Clostridium butyricum* on growth, intestine health status and resistance to ammonia stress in Pacific white shrimp *Litopenaeus vannamei*[J]. Fish & Shellfish Immunology, 2017, 65: 25-33.
[33] 刘小华, 李舒梅, 熊跃玲. 短链脂肪酸对肠道功效及其机制的研究进展 [J]. 肠外与肠内营养, 2012, 19(1): 56-58.
[34] 占秀安, 胡彩虹, 许梓荣. 果寡糖对肉鸡生长、肠道菌群和肠形态的影响 [J]. 中国兽医学报, 2003, 23(2): 196-198.
[35] 王继成, 潘灵辉, 李淑云, 等. 低聚木糖对断奶仔猪生产性能、肠道菌群及免疫水平影响的研究 [J]. 中国畜牧兽医, 2006, 33(5): 3-7.
[36] SUO H Q, LIN L U, XU G H, et al. Effectiveness of dietary xylo-oligosaccharides for broilers fed a conventional corn-soybean meal diet[J]. Journal of Integrative Agriculture, 2015, 14(10): 2050-2057.
[37] 刘卫东, 宋素芳, 程璞. 甘露寡糖和益生菌对肉仔鸡生产性能和肠道菌群的影响 [J]. 家畜生态学报, 2011, 32(1): 32-35.
[38] 温若竹, 江芸, 刘泽兴, 等. 甘露寡糖对肉仔鸡肠道微生物区系发育的影响 [J]. 浙江大学学报: 农业与生命科学版, 2011, 37(1): 83-90.
[39] 梁超. 饲料中添加二甲酸钾、低聚木糖和菊粉对刺参的生长、免疫和抗灿烂弧菌感染能力的影

响 [D]. 硕士学位论文. 青岛: 中国海洋大学, 2011.
[40] 盛清凯, 姚惠源. 低聚糖对肠道菌群的调节机理 [J]. 动物科学与动物医学, 2002, 19(2): 35-38.
[41] PESTOVA M I, CLIFT R E, VICKERS R J, et al. Effect of weaning and dietary galactose supplementation on digesta glycoproteins in pigs[J]. Journal of the Science of Food and Agriculture, 2000, 80(13): 1918-1924.

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