Front. Agric. China 2011, 5(4): 612–617
DOI 10.1007/s11703-011-1129-8

RESEARCH ARTICLE

Effects of xylooligosaccharide on growth performance, activities of digestive enzymes, and intestinal microflora of juvenile *Pelodiscus sinensis*

Yueqiang GUAN (✉), Huan ZHOU, Zhili WANG

School of Life Sciences, Hebei University, Baoding 071002, China

Abstract **The effects of xylooligosaccharide (XOS) on growth performance, activities of digestive enzymes, and intestinal microflora of Chinese soft-shelled turtle *Pelodiscus sinensis* were investigated by adding different concentrations of XOS in the diet of juvenile *P. sinensis*. The turtles with an average initial bodyweight of 12.26± 0.32 g were randomly divided into five groups, which were fed with diets containing 0 mg/kg (control), 50 mg/kg (Trial I), 100 mg/kg (Trial II), 200 mg/kg (Trial III), and 500 mg/kg (Trial IV) XOS product (95%), respectively. The feeding trial lasted for 30 days. Indices described above were determined at the end of the experiment. The results showed that the diet supplemented with XOS could enhance the growth of *P. sinensis*, and the growth rate of Trial III was the highest. Feed conversion ratio (FCR) of the trials was lower than that of the control. All the trials had higher activities of intestinal digestive enzymes than the control. The amylase activity of Trial II was the highest and was significantly different from that of the control, while the protease activity of the trials was not significantly but slightly higher than that of the control. The numbers of total aerobic bacteria and Enterobacteria of all the trials were significantly lower than those of the control. The number of Bifidobacteria of Trial II ranked the largest, significantly higher than that of the control, whereas the number of Bifidobacteria of all the other trials was higher but not more significant than that of the control. The results demonstrated that XOS supplementation in the diet of *P. sinensis* could enhance its growth and decrease FCR. Furthermore, it could enhance activities of intestinal protease and amylase and optimize its intestinal microflora. The recommended supplemental concentration of XOS in the diet of Juvenile *P. sinensis* should be 100–200 mg/kg.**

Keywords *Pelodiscus sinensis*, xylooligosaccharide, specific growth rate, digestive enzyme, microflora

Introduction

Chinese soft-shelled turtle *Pelodiscus sinensis* is an important freshwater species in China and some Southeast Asian countries, such as Malaysia, Vietnam, and Indonesia, with high nutritional and economic values (Chu et al., 2007). With the development of its culture, various kinds of bacterial diseases have been broken out (Shen et al., 2000). To prevent and control the outbreak of bacterial disease, antibiotics has become commonly used and the kinds of antibiotics and the dosages are ever increasing. However, the adverse effects associated with the using of antibiotics in aquaculture are notorious (Qi et al., 2009). Antibiotics abuse will produce more drug-tolerant bacteria strains and lead to unbalanced microflora. Aquatic bacterium can also develop resistance genes as a consequence of exposure to antimicrobial agents. Also, the occurrence of antimicrobial residues in products of aquaculture threatens human health. The use of probiotics (beneficial microbiota consumed by animals) and prebiotics (food for indigenous microbiota in the gastrointestinal tract), which control pathogens through a variety of mechanisms, is increasingly viewed as an alternative to antibiotic treatment. Xylooligosaccharide (XOS) is an important prebiotics and

Received May 9, 2011; accepted June 13, 2011
Correspondence: Yueqiang GUAN
E-mail: guanyueqiang@hbu.edu.cn

mostly widely used with the advantage of highly stability at acid or high-temperature conditions. It consists of 2–7 xylose molecules combined with β-(1–4) glucosidic bond. It is hardly digested in the gastrointestinal tract of the animals; meanwhile, it can multiply Bifidobacteria with high selectivity. Xiong (2005) discovered that weight gain (WG) of allogynogenetic crucian carp *Carassais auratus gibebio* increased significantly after fed with XOS-supplemented diet. Similarly, WG of grass carp *Ctenopharyngodon idellus* increased after fed with XOS-supplemented diet (Chu et al., 2008). Until now, few reports about the effect of XOS on *P. sinensis* have been published. To provide suggestions to the healthy culture and utilization of XOS in the feed of *P. sinensis*, effects of XOS on the growth performance, digestive enzyme activities, and intestinal microflora of juvenile *P. sinensis* were studied.

Materials and methods

Experimental animals and ration

Experimental animals were obtained from the farm of Xushui County, Hebei Province, China. After they were brought into the laboratory, the animals were acclimated to the aquaria (50 cm × 50 cm × 22 cm). Each aquarium contains three turtles. The depth of the water in each aquarium was 7–8 cm and the temperature was maintained at (31±0.5)°C. Natural sunlight and artificial light were provided. 20% of the water was replaced by aerated tap water at the same temperature at 08:00 everyday. The turtles were fed twice a day (08:30 and 17:00). Juvenile turtle's commercially formulated diet (Haitai Co., Ltd., China) was selected as the basal diet with the main nutrients listed in Table 1. The experiment was carried out after acclimation for 10 days by mixing the diet with warm water and then twisting it into a 1 cm length strip. Several feed sites were set in each tank in order to assure that the diet was available to each turtle. The feeding rate was 3%.

Experimental design

Ninety healthy and active juvenile turtles were chosen after the acclimation period. The weight of each turtle was measured after 1 day fasting and the initial average weight was 12.26±0.32 g. The turtles were randomly distributed into five triplicate groups, which were fed with diets containing 0 mg/kg (control), 50 mg/kg (Trial I), 100 mg/kg (Trial II), 200 mg/kg (Trial III), and 500 mg/kg (Trial IV) XOS product (Shandong Longli Biotechnology Co., Ltd.). The concentration of XOS in the product was 95%. The experiment lasted 30 days.

Measurement of growth performance indices

After the feeding experiment, the experimental juvenile turtles were fasted for 1 day in order to avoid the effect of remaining diet on the intestinal digestive enzyme activity and the final weight of each turtle was measured. WG, specific growth rate (SGR), and feed conversion ratio (FCR) were determined according to the following equations:

$$WG(\%) = [(W_t - W_0)/W_0] \times 100;$$

$$SGR(\%) = [(\ln W_t - \ln W_0)/t] \times 100;$$

$$FCR = \text{Diet Consumed}/(W_t - W_0),$$

where W_0, W_t, and t were initial bodyweight, final bodyweight, and experimental duration (days), respectively.

Measurement of activities of intestinal digestive enzymes

All the experimental turtles were killed and dissected at the end of the experiment. After the intestine was taken and the fat was removed, the intestine was washed in cold distilled water. Each turtle's intestine was cut into two halves horizontally. Part 1 was used to measure the activities of digestive enzyme and Part 2 was used to study microflora of intestine.

Part 1 was put into the liquid nitrogen for 2 min and then kept at −80°C until measuring the activities of digestive enzyme. The intestine was unfrozen at 4°C and weighted. The tissues were homogenized in 10 volumes (v/w) ice-cold distilled water. The homogenate was centrifuged at 3600 r/min under 4°C for 15 min. The supernatant was kept at 4°C and assayed for the activity of digestive enzyme. The protein concentration of supernatant was measured according to Bradford (1976) using bovine serum albumin as a standard. Amylase activity was measured according to iodine starch method (Medicinal Assay Institute of Shanghai, 1979) using iodine solution to reveal non-hydrolyzed starch. Protease activity was measured according to Lowry et al. (1951) using Folin-phenol reagent. Enzymatic activities were both expressed as specific activity (U/mg protein).

Measurement of microflora in the intestine

Part 2 was previously kept at 4°C; then, the proposed bacteria were isolated and cultured in order to analyze microflora of intestine, which was rapidly homogenized with the homogenate diluted and coated on eosin methylene blue (EMB) agar media, aerobic bacteria media, and modified BS media, in order to culture Enterobacteria, total aerobic bacteria, and

Table 1 Main nutrient of the basic diet (air dry basis)

Item	Crude protein	Crude fat	Crude fiber	Crude ash	Calcium	Phosphorus	Total amino acids
Percent	41%	4.5%	1.5%	16%	2%–5%	1.2%	37%

The data were supplied by Haitai Ltd.

Bifidobacteria, respectively (Xiong et al., 1994; Yang et al., 2005). Among them, Enterobacteria and total aerobic bacteria were cultured at 31°C for 24 h, while Bifidobacteria were cultured at 31°C for 72 h under anaerobic condition and the numbers of proposed bacterium were counted with the method of standard plate colony expressed as colony-forming units (CFU)/g intestine tissue.

Statistical analysis

The data were analyzed by statistical software SPSS13.0. The differences among treatments were analyzed using one-way ANOVA. When the homogeneity of variances existed, the difference between treatments was further analyzed using Duncan's multiple comparison tests. The results were expressed as mean±S.D. at $P < 0.05$.

Results

Effect of XOS supplementation on growth performance of juvenile *P. sinensis*

Survival rate, WG, SGR, and FCR of juvenile *P. sinensis* after fed with diet containing XOS are listed in Table 2. At the end of the experiment, survival rate of all the treatment was 100%, i.e., all the experimental animals were alive at the end of the experiment. WG of the four trials was all higher than that of the control. Among them, WG of Trial III was the highest. SGR of each trial was higher than that of the control and the SGR of Trial III was also the highest. FCR of each trial was lower than that of the control. However, the FCR of Trial III was the lowest. In conclusion, supplementation of XOS at 200 mg/kg showed the best according to WG and FCR.

Effect of XOS supplementation on the activities of intestinal amylase and protease

Effects of XOS supplementation on amylase and protease activities are shown in Table 3. The differences of amylase activity among the treatments were significant ($F = 4.346 > F_{0.05}$ (4,16), $P < 0.05$), and the activity of each trial was higher than that of the control. Among all the trials, the activity of Trial II and Trial IV was significantly higher than that of the control ($P < 0.05$); the differences of protease activity among the treatments were not significant ($F = 0.524 < F_{0.05}$ (4,16), $P > 0.05$), but protease activity of each trial was higher than that of the control, and the activity of Trial III ranked the highest.

Effect of XOS on the microflora of the intestinal tract

Effects of XOS on the intestinal microflora are shown in Table 4. It indicates that the number of total aerobic bacteria in each trial was significantly lower than that of the control ($F = 58.191 > F_{0.05}$ (4, 16), $P < 0.05$), and Enterobacteria in each trial was significantly lower than that of the control ($F = 107.709 > F_{0.05}$ (4, 16), $P < 0.05$). In the meantime, the number of Bifidobacteria in the trials was significantly higher than that of the control ($F = 27.804 > F_{0.05}$ (4, 16), $P < 0.05$), and among the trials, the number of Trial III was significantly higher than that of the control.

Discussion

Effect of XOS on growth performance of juvenile *P. sinensis*

Zhang (2003) investigated the effect of different concentrations of a dietary mannan-oligosaccharides (MOS) in Jian carp *Cyprinus carpio* var. *Jian*, reported improved WG, FCR, and productivity, and finally concluded for juvenile Jian carp that the optimum amount of MOS in the diet was 0.3%. Li (2004) studied similarly and also demonstrated the promotion effect of grow performance after MOS administration. Xiao et al. (2004a) reported that, when fed with diet supplemented with fructooligosaccharides (FOS) and saccharicterpenin, the growth performance of juvenile Chinese soft-shelled turtle *P. sinensis* increased and the feed coefficient decreased.

Table 2 Effects of dietary XOS on growth performance of *P. sinensis*

Item	Control	Trial I	Trial II	Trial III	Trial IV
Survival rate (%)	100	100	100	100	100
WG (%)	70.52±0.56	84.92±0.38	80.37±0.67	97.41±1.43	73.87±0.49
SGR (%)	1.78±0.05	2.05±0.04	1.97±0.01	2.27±0.10	1.84±0.08
FCR	2.10±0.07	1.81±0.08	1.89±0.04	1.63±0.12	2.02±0.06

Value = Mean±S.D., $n = 3$.

Table 3 Effects of dietary XOS on activities of intestinal enzymes of *P. sinensis* (U/mg protein)

Item	Control	Trial I	Trial II	Trial III	Trial IV
Amylase	3.26 ± 0.18^b	3.43 ± 0.05^b	3.63 ± 0.15^a	3.36 ± 0.13^b	3.50 ± 0.12^a
Protease	1.71 ± 0.52^a	1.89 ± 0.48^a	1.94 ± 0.31^a	2.20 ± 0.55^a	1.80 ± 0.63^a

In the same line, values with same superscript lowercase letters were not significantly different ($P > 0.05$). $n = 4$.

Table 4 Effects of dietary XOS on intestinal microflora of *P. sinensis*

Item	Control	Trial I	Trial II	Trial III	Trial IV
Total aerobic bacteria ($\times 10^4$ CFU/g)	487.5 ± 88.5^{a}	47.5 ± 7.8^{c}	158.8 ± 59.3^{b}	51.8 ± 13.2^{c}	53.3 ± 26.7^{c}
Enterobacteria ($\times 10^4$ CFU/g)	337.5 ± 35.0^{a}	69.0 ± 13.2^{cd}	150.8 ± 28.5^{b}	85.5 ± 21.1^{c}	36.5 ± 4.7^{d}
Bifidobacteria ($\times 10^4$ CFU/g)	59.3 ± 19.0^{b}	37.0 ± 9.0^{b}	437.5 ± 126.6^{a}	82.5 ± 38.6^{b}	78.8 ± 48.0^{b}

In the same line, values with same superscript lowercase letters were not significantly different ($P > 0.05$). $n = 4$.

Among all the treatments, the group with 1000 mg/kg FOS and 1000 mg/kg saccharicterpenin performed the best. Ji et al. (2004) indicated that WG and survival rate of juvenile Chinese soft-shelled turtle *P. sinensis* were significantly enhanced after supplementation with 0.25% FOS in the diet. Staykov et al. (2007) reported that MOS supplementation in the diet of rainbow trout *Oncorhynchus mykiss* increased WG, reduced FCR and mortality, and improved indicators of immune status in net cages or raceways. Wang et al. (2008) discovered that supplementation of galacto-mannan-oligo-saccharides in the diet of juvenile allogynogenetic crucian carp *C. auratus gibelio* could increase WG and SGR of the fish. Li et al. (2008) reported that SGR and FCR of juvenile turbot *Scophthalmus maximus* were significantly enhanced, when fed with yeast cell wall, XOS, and the combined supplemented diet for 72 days. In the present study, it showed a promotive effect of growth performance of juvenile *P. sinensis* fed with XOS-supplemented diet. Among the trials, Trial III showed the best growth performance, which was significantly higher than the control. These results indicated that supplementation of XOS or some other oligosaccharides in the diet could enhance growth of aquatic animal.

Effect of XOS on intestinal digestive enzyme activities in juvenile *P. sinensis*

In our experiment, activities of amylase and protease were both enhanced. Xiao et al. (2004b) reported activities of digestive enzyme of juvenile *P. sinensis* were enhanced by diet supplemented with FOS and saccharicterpenin. Liu et al. (2004) reported that supplementation of *Bacillus licheniformis* and XOS could promote amylase activities of chyme in the midgut and protease activity in hepatopancreas of allogygenetic crucian carp *C. auratus gibelio*. Chen et al. (2006) reported that activities of protease and amylase of *C. auratus gibelio* were enhanced when fed with diet supplemented with 0.75% or 1% chitosan-oligosaccharide. Xu et al. (2009) reported that activities of digestive enzyme of *C. auratus gibelio* increased after feeding on the XOS supplemented diet.

All these reports about XOS or other oligosaccharides could enhance activities of digestive enzymes. It might be due to oligosaccharides that could proliferate probiotics such as Bifidobacteria or *Lactobacillus*; then, the probiotics and their metabolites could stimulate the production of digestive enzymes and vermiculation of the intestine. Furthermore, the produced probiotics could actively produce a range of relevant enzymes such as amylase, protease, and lipase (Sáenz de Rodrigáñez et al., 2009). Besides the stimulation of digestive enzymes, prebiotics can enhance proliferation of epithelial cells and stimulate the development of intestinal tracts (Howard et al., 1995). Zhou et al. (2009) also reported that the microvilli length in pyloric ceca, proximal, and mid-intestine of red drum *Sciaenops ocellatus* was significantly increased by the supplementation with prebiotics. The stimulation of digestive enzymes and the better development of intestinal tracts altogether caused by prebiotics might promote the absorption and digestion of the nutrients consequently.

Effect of XOS on intestinal microflora of *P. sinensis*

Prebiotics beneficially affected the host by selectively stimulating the growth and/or activity of one or limited number of bacterial species already resident in the colon. Intake of prebiotics could significantly modulate the colonic microbiota by increasing the number of specific bacteria and thus changing the composition of the microbiota (Gibson and Roberfroid, 1995). The present results indicated that the number of total aerobic bacteria and Enterobacteria decreased, whereas the number of Bifidobacteria significantly increased. In detail, the reason might be that XOS could increase the population of Bifidobacteria and other beneficial microorganisms and they would occupy the adhesion sites in the intestine and inhibit the adhesion of pathogenic bacteria in the intestine through competitive expulsion.

There are some other reports about prebiotics to improve the intestinal microflora of aquatic animals. For example, the number of *Escherichia coli* in the gut of carp *C. carpio* decreased significantly, whereas the number of Bifidobacteria increased significantly after feeding on diet supplemented with MOS (Zhang, 2003). The number of *E. coli* decreased by 10% and that of *Lactobacillus lactis* increased in the gut of *C. auratus gibebio* after being fed with 0.1% XOS and 0.1% *Baclicus lincheniformis*-supplemented diet (Xiong, 2005). The number of aerobic bacteria in the intestine of *Litopenaeus vannamei* significantly decreased by diet supplemented with 0.02% XOS (Hu, 2007). Previda™ (containing galacto-glucomannans from hemicellulose extract) supplementation in the diet of red drum resulted in significant improvements in growth performance and immunological responses (Zhou et al., 2009).

Besides aquatic animal, prebiotics also have a great effect on the land mammals and human beings. For example, both XOS and FOS supplementation in the diet of rat markedly increased the Bifidobacteria population and XOS had a greater effect on the bacterial population than FOS (Hsu et al., 2004). XOS supplementation significantly increased the population of Bifidobacteria and the fecal moisture content but decreased the fecal pH value in the elderly (65 years and older) after 3 weeks' administration, indicating that it was effective in promoting the intestinal health without adverse effects on nutritional status in the elderly (Chung et al., 2007). All these studies indicated that supplementation with XOS or some other prebiotics could affect intestinal microflora greatly.

Conclusions

WG of juvenile *P. sinensis* increased while FCR decreased when fed with diet containing XOS. Activities of amylase and protease in the intestine were enhanced by diet containing XOS. The numbers of total aerobic bacteria and Enterobacteria were significantly decreased, whereas the number of Bifidobacteria decreased after feeding on diet containing XOS. In practice, the optimal concentration of XOS in the diet of juvenile *P. sinensis* should be 100–200 mg/kg in the diet.

Acknowledgements

This research was supported by the Natural Science Foundation of Hebei Province, China (No. C2010000253) and National Programs for Science and Technology Development of Baoding, China (No. 09ZF003).

References

Bradford M M (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 72(1–2): 248–254

Chen Y, Zhou H Q, Leng X J, Zhong G F (2006). Effects of chitosan on growth and digestive enzyme in allogynogenetic silver crucian carp. Journal of Fishery Sciences of China, 13(3): 440–445 (in Chinese)

Chu J H, Chen S M, Huang C H (2007). Effect of dietary iron concentrations on growth, hematological parameters, and lipid peroxidation of soft-shelled turtles, *Pelodiscus sinensis.* Aquaculture, 269(1–4): 532–537

Chu W Y, Wu X, Cheng J, Fu G H, Liu Z, Zhang J S (2008). Effect of xylooligosaccharide on growth performance and blood biochemical indices of *Ctenopharyngodon idellus.* Feed Research, 6: 60–61 (in Chinese)

Chung Y C, Hsu C K, Ko C Y, Chan Y C (2007). Dietary intake of xylooligosaccharides improves the intestinal microbiota, fecal moisture, and pH value in the elderly. Nutr Res, 27(12): 756–761

Gibson G R, Roberfroid M B (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr, 125 (6): 1401–1412

Howard M D, Gordon D T, Garleb K A, Kerley M S (1995). Dietary fructooligosaccharide, xylooligosaccharide and gum Arabic have variable effects on cecal and colonic microbiota and epithelial cell proliferation in mice and rats. J Nutr, 125(10): 2604–2609

Hsu C K, Liao J W, Chung Y C, Hsieh C P, Chan Y C (2004). Xylooligosaccharides and fructooligosaccharides affect the intestinal microbiota and precancerous colonic lesion development in rats. J Nutr, 134(6): 1523–1528

Hu Y (2007). Optimum feed formula selection and some feed additive application on juvenile *Litopenaeus vannamei*. Dissertation for the Doctoral Degree. Qingdao: China Ocean University, 128–144 (in Chinese)

Ji G H, Liu Z Z, Leng X J (2004). Effects of dietary betaglucan and fructooligosaccharides on the growth and activities of superoxide dismutase and lysozyme of *Trionyx sinensis.* Journal of Shanghai Fisheries University, 13(1): 36–40 (in Chinese)

Li Y, Wang Y J, Wang L, Jiang K Y (2008). Influence of several non-nutrient additives on nonspecific immunity and growth of juvenile turbot, *Scophthalmus maximus* L.. Aquacult Nutr, 14(5): 387–395

Li Y L (2004). The effect of mannanoligosaccharide (MOS) on the intestinal microflora and immune function of juvenile Jian Carp *Cyprinus carpio* var. *jian*. Dissertation for the Master's Degree. Ya'an: Sichuan Agricultural University: 19–28 (in Chinese)

Liu B, Xie J, Liu W B, Wang T, Wang H F, Du W (2004). Effects of *Bacillus licheniformis* and xylooligosaccharides on digestive enzyme activities, growth and microflora in intestine in allogygenetic crucian carp. Journal of Dalian Fisheries University, 21(4): 336–340 (in Chinese)

Lowry O H, Rosebrough N J, Farr A L, Randall R J (1951). Protein measurement with the Folin phenol reagent. J Biol Chem, 193(1): 265–275

Qi Z Z, Zhang X H, Boon N, Bossier P (2009). Probiotics in aquaculture of China- current state, problems and prospect. Aquaculture, 290(1–2): 15–21

Sáenz de Rodrigáñez M A, Díaz-Rosales P, Chabrillón M, Smidt H, Arijo S, León-Rubio J M, Alarcón F J, Balebona M C, Moriñigo M A, Cara J B, Moyano F J (2009). Effect of dietary administration of probiotics on growth and intestine functionality of juvenile Senegalese sole (*Solea senegalensis*, Kaup 1858). Aquacult Nutr, 15(2): 177–185

Shen J Y, Yin W L, Qian D, Cao Z, Shen Z H (2000). Studies on the immunization of bacterin against bacterial diseases of cultivated soft-shelled turtle. Journal of Zhejiang University (Agric. & Life Sci.), 26 (3): 325– 328 (in Chinese)

Staykov Y, Spring P, Denev S, Sweetman J (2007). Effect of a mannan oligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*). Aquacult Int, 15(2): 153–161

Medicinal Assay Institute of Shanghai (1979). Clinical Biochemistry Assay. Shanghai: Shanghai Science and technology Press, 366–368 (in Chinese)

Wang R, Liu J, Liu H Y, Shuai J Y (2008). Effect of dietary galactomannan oligosaccharides on growth performance and non-specific immune responses of juvenile gibel carp (*Carassius auratus gibelio*). Journal of Shanghai Fisheries University, 17(4): 502–506 (in Chinese)

Xiao M S, Wang Z G, Cui F, Hong Y Z (2004a). Orthogonality experiment on effects of fructooligosaccharide and saccharicterpenin on the growth of turtles. Freshwater Fisheries, 34(3): 13–15 (in Chinese)

Xiao M S, Wang Z G, Sun Y J, Tang S Y (2004b). Effects of feed supplemented with fructooligosaccharide and saccharicterpenin on the activities of digestive enzymes of turtles. Chinese Animal Husbandry and Veterinary Medicine, 13(2): 10–13 (in Chinese)

Xiong D X, Wu Z Q, He L J (1994). Clinical anaerobic bacteria detection manual. Beijing: Chinese Science and Technology Press, 60–87 (in Chinese)

Xiong S X (2005). Effects of Xylooligosaccharides in diets on the weight gain ratio, digestive enzyme activity and immune performance of Allogynogenetic Crucian Carp. Dissertation for the Master's Degree. Nanjing: Nanjing Agricultural University, 29–55(in Chinese)

Xu B H, Wang Y B, Li J R, Lin Q (2009). Effect of prebiotic xylooligosaccharides on growth performances and digestive enzyme activities of allogynogenetic crucian carp (*Carassius auratus gibelio*). Fish Physiol Biochem, 35(3): 351–357

Yang J X, Zhang X, Cai J P, Wang S (2005). Studies of enzyme producing bacteria strains in the gut of *Tilapia nilotica*. Water conservancy related fisheries, 25(3):10–18 (in Chinese)

Zhang H M (2003). Effect of mannan-oligosaccharide on the production performance and biological indicator of carps. Dissertation for the Master's Degree. Baoding: Hebei Agricultural University, 15–36 (in Chinese)

Zhou Q C, Buentello J A, Gatlin D M (2009). Effects of dietary prebiotics on growth performance, immune response and intestinal morphology of red drum (*Sciaenops ocellatus*). Aquacult Nutr, 309 (1–4): 253–257