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Full Length Article

The Influences of Dietary Supplementation with Fructooligosaccharide on Growth and Immune Responses of Climbing Perch (*Anabas testudineus*)

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Abstract

The effects of prebiotic-fructooligosaccharide (FOS) on growth performances, lysozyme and phagocytosis activities of Climbing perch (*Anabas testudineus* Bloch, 1792) were determined. The factorial completely randomized experiment (2 × 3) was applied. Three experimental diets were formulated to contain 0, 1, and 2% FOS in pellet diets with two feeding levels including either 3% of body weight or satiation. Fish (mean body weight of 9.33±0.06 g) had been raised for 120 days. The results showed fish fed 2% FOS supplementary diet at 3% of body weight and 0, 1, 2% FOS diets at satiation gained greater body weight, specific growth rate, and increased weight than perch received a feed without FOS at 3% of body weight ($P<0.05$). Moreover, climbing perch obtained feeds with 1 and 2% FOS at 3% of body weight and satiation had better daily feed intake and total feed intake ($P<0.05$). Furthermore, fish received diets with 1 and 2% FOS at 3% of body weight and satiation had significantly higher ($P <0.05$) lysozyme activity and percent phagocytosis than fish fed without FOS diet both two feeding levels. In sum, the 1% prebiotic-FOS supplemented diet with satiation feeding can improve growth performances and enhance non-specific immune responses of Climbing perch, also influence the total production and feed cost. © 2017 Friends Science Publishers

Keywords: Climbing perch; Prebiotic; Fructooligosaccharide; Fish immunity

Introduction

Climbing perch (*Anabas testudineus*) is a commercially important fish cultured in Southeast Asia including Thailand and Vietnam and was introduced into Bangladesh in 2012. Because of their labyrinth organ, placed behind the gills, they are able to tolerate low dissolved oxygen. As a result, most farmers are able to stock this fish in very high density. However, several studies showed the negative effects of too dense density on chronic stress, growth reduction, and fish health deterioration.

To improve fish health and prevent them from serious pathogens, non-specific immunity improvement is one of the most promising alternative tools to combat with severe diseases as well as reduce the antibiotic and chemical application which possibly causes hazardous residues in consumers and aquatic environment. Considerable immunostimulants including chitin, chitosan, glucan, herbs, vitamins, prebiotics, and probiotics have been evaluated and applied in land animal and fish farms. Prebiotics are not digested but these act as immunostimulants to enhance fish phagocytosis (Chitmanat, 2002) and improve growth performances. They are able to clearly regulate microorganisms in a gastrointestinal tract by boosting beneficial bacteria number (Gibson and Roberfroid, 1995).

Fructooligosaccharides (FOS) is one of the most promising prebiotics which can be used as protective and eco-friendly substitutes to antibiotics in fish culture. FOS is short chain of β-D-fructans presenting in various foods (Fuller and Gibson, 1998). However, the application of FOS in fish is rather new. These were studied in turbot (*Psetta maxima*) (Mahious *et al*., 2006), sea cucumber (*Apostichopus japonicus*) (Zhang *et al*., 2010), blunt snout bream (*Megalobrama amblycephala*) (Zhang *et al*., 2014), gilthead sea bream (*Sparus aurata*) (Guerreiro *et al*., 2016), Pacific white shrimp (*Litopenaeus vannamei*) (Zhou *et al*., 2007). All found that FOS effectively improved growth performances and boosted immune responses.

The suitable dosage for an application of FOS varies from species to species and fish age as well as culture environments. The aim of this research was to investigate the influences of FOS on growth performances and immune responses in Climbing perch (*Anabas testudineus*). The obtained information possibly provides valuable instructions for the application of prebiotics and reduction in antibiotic usage in commercial fish culture.

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Materials and Methods

Experimental Diets

The commercial pellet feeds containing approximately 30% protein were supplemented with 0, 1, or 2% (of dry weight) Fructooligasaccharide, FOS. The experimental feeds were then dried and kept at 4°C. Fish in a control group were fed with diet without FOS supplementation. The experimental feed proximate composition was examined as stated in the AOAC methods (2005) and was reported in Table 1.

Feeding and Culture System

Climbing Perch juveniles were obtained from a local private hatchery and nursery (Chiangmai, Thailand). They had stocked in the cage (2 x 2 x 1.5 m^3) for 14 days before the experiment was started to acclimate fish. A control feed had been applied twice a day in the course of the acclimation. Later, fish with an average body weight of 9.33±0.06 g were put in 18 cages (2 x 2 x 1.5 m^3) at random, with 30 fish in each cage.

A factorial (2X3) experiment was carried out in order to investigate the interaction between Fructooligasaccharide supplementation (0, 1 or 2%) and feeding rate (3% of body weight or satiation) on growth performances, lysozyme and phagocytosis activities. Each treatment consisted of three replicates. The cages were set in $1{,}000$ m^2 earthen pond. Fish were fed on the experimental diet for 120 days either 3% of body weight or satiation, across 2 feeding times (09:00 and 15:00). Feeding was adjusted on the basis of growth every 30 days.

Growth Performances, Data Collection and Analysis

After fish had been anesthetized, they were randomly weighed every 15 days to analyze the growth indices. At the end of an experiment, the following formulas were used to calculate growth performances:

Weight gain = Wt – W0

Specific growth rate = (Ln Wt – Ln W0) X 100/t

Feed conversion ratio (FCR) = experimental feed consumed (g)/weight gain (g)

Survival rate = (Nt – N0) X 100

Wt: final weight (g), W0: initial weight (g),

t: experimental trial period (day),

N0: the number of fish stocked at the beginning,

Nt: the remained fish at the end.

Blood Sample Collection and Immunity Determination

At the end of the feeding trial, 3 fish were randomly sampled from each replicates and were then anaesthetized. Fish were starved for 24 h before approximately two mL of blood was taken from the caudal vein. Blood spinning in non-heparinized tubes were conducted at 1000 g for five min and kept at –20ºC for lysozyme activity examination.

Lysozyme activity: The determination of lysozyme activity was conducted according to Puangkaew *et al.* (2004) method depending on the lysis of *Micrococcus lysodeikticus*, a Gram-positive bacterium (Sigma). A 25 µL of serum sample was mixed to 175 µL of 0.3 mg mL^{-1} *M. lysodeikticus* solution in 0.1 M phosphate buffer, pH 6.8. Results were calculated in units of lysozyme mL^{-1} serum. One unit is described as the amount of sample causing a decrease in absorbance of 0.001 min^{-1} at 450 nm. *M. lysodeikticus* suspension without serum was used as the control.

Phagocytic activity: The phagocytosis activity was assayed using a modification of the method of Zhang *et al*. (2014). 200 µL leucocyte suspensions (2×10^6 cells mL^{-1}) were dispersed over cover slips and incubated at 25ºC for two hours. Cells were later washed with RPMI 1640 to remove non-adherent cells. 100 µL of yeast suspension (Sigma) solution 1×10^8 cells mL^{-1} were spread on cover slips and left at room temperature for an hour. After that, the non-phagocyte yeast cells were washed with RPMI 1640. The adherent cells were fixed with methanol and stained by Diff-Quick staining dye (Sigma). The number of leukocytes that engulfed yeast 200 adhered cells was counted. The phagocytic index (PI) was calculated as follows: PI = number of phagocytic cells with engulfed yeast divided by total phagocytic cells.

Water Quality Maintenance

Water temperature, dissolved oxygen, and pH were measured by an YSI model 59 multi-probe meter (Geotech, USA). Ammonia and Nitrite nitrogen were examined by Phenol and Coupling Methods, respectively. Water temperatures were between 25 and 28ºC, pH ranged from 7.5 to 7.8 and dissolved oxygen was continuously aerated above 5.0 mg L^{-1}.

Statistical Analysis

Design of the experiments was factorial completely randomized experiment (2 × 3) with three replications. The results were analyzed using Duncan's multiple range tests by using the Social Sciences (SPSS) to compare the mean differences among treatments ($p>0.05$).

Results

Growth Performances and Survival

Fish received FOS prebiotic supplementary feeds improved growth performances (Table 2); however, no significant differences in the final weight, specific growth rate, feed conversion ratio, and survival rate of climbing perch fed with different levels of FOS supplementary feeds. Compared to the control treatment, fish received on 2%

Table 1: chemical proximate composition of the experimental diets

FOS (%)	Moisture (%)	(% dry weight basis)				
		protein	lipid	Nitrogen-free extracts (NFE)*	fiber	ash
0	7.92±0.06	29.51±2.21	9.04±0.56	46.47±1.65	4.43±1.11	10.55±0.03
1	7.12±0.06	30.96±0.76	8.87±0.66	45.19±0.13	4.52±0.40	10.46±0.08
2	7.95±0.67	29.58±0.55	10.24±0.48	45.14±1.11	4.71±0.56	10.32±0.10

*****Nitrogen-free extracts (NFE) = dry matter - (crude protein + crude lipid + ash + fiber)

Table 2: Growth performances of Climbing perch fed the diets with different levels of fructooligosaccharide (FOS) at two feeding levels, 3% of weight or satiation

Feeding rate	FOS level (%)	Final weight (g/fish)	Increased body weight (g/fish)	Specific growth rate (%/d)	Feed conversion ratio	Survival (%)
	0	38.51 ± 1.32^{a}	29.15 ± 1.27^{a}	1.18 ± 0.02^{a}	2.23±0.08	87.27±1.05
3% of body weight	1	40.22 ± 1.18^{ab}	30.88 ± 1.17^{ab}	1.21 ± 0.02^{ab}	2.34±0.09	88.48±0.60
	2	42.43 ± 1.10^{bc}	33.16 ± 1.08^{bc}	1.26 ± 0.02^{bc}	2.17±0.08	87.88±3.03
	0	43.13 ± 0.97^{bc}	33.75 ± 0.98^{bc}	1.27 ± 0.01^{bc}	2.18±0.03	86.66±0.60
Satiation	1	45.73 ± 0.48^{c}	36.42 ± 0.48^{c}	1.32 ± 0.00^{c}	2.06±0.00	87.87±0.60
	2	41.88 ± 0.95^{b}	32.54 ± 0.98^{b}	1.25 ± 0.02^{b}	2.29±0.15	86.06±2.64
Two - way ANOVA						
Feeding rates		*	*	*	ns	ns
Prebiotic levels		ns	ns	ns	ns	ns
interaction		*	*	*	ns	ns

Means ± SE with different superscripts in the same column are significantly different (P<0.05)
ns represents not significance (P > 0.05), while * is significant different (P < 0.05)

FOS supplemented diet significantly improved final weight, upsurge body weight, and specific growth rate (P < 0.05) at 3% body weight feeding. They were not significant differences in feed conversion ratio and survival rate (P>0.05). Nonetheless, the interaction between feeding rates and FOS levels was noticeable in growth performances but not in feed conversion ratio and survival rates. The best growth performances were observed in climbing perch with satiation feeding 1% FOS addition.

Immune Parameters

Immune parameters including serum lysozyme and phagocytosis activities of Climbing Perch are shown in Table 3 and 4, respectively. Supplementation with the FOS prebiotic enhanced lysozyme and phagocytosis activities of fish. When feeding to satiation, the serum lysozyme activity of fish was improved by dietary 1–2% FOS (P < 0.05). There was no significant difference between the 1 and 2% FOS groups (P > 0.05) (Table 3). Prebiotic levels affected the lysozyme and phagocytosis activities, while there were no influences of feeding rates as well as the interaction between FOS levels and feeding rates on these immune parameters.

Discussion

Fish received the FOS supplemented feeds resulted in better growth performances, improved lysozyme and phagocytosis activities. Stanton *et al.* (2005) stated that feeding prebiotics could enhance ready-to-use valuable nutrients which fish are able to use immediately and subsequently promote their growth. Some FOS products result in the digestion of nutrients in fish which could possibly be associated with a growth promotion (Renjie *et al*., 2010). Digestive enzyme improvement could be likely related to prebiotic fermentation by endogenous gut microorganisms (Soleimani *et al*., 2012).

The application of prebiotics in aquatic animals gained benefits on growth (Guerreiro *et al*., 2015), gut microbiota (Ricke, 2005; Zhou *et al*., 2010), resistance against pathogenic bacteria (Talpur *et al*., 2014) and innate immune parameters including alternative complement activity (ACH50) (Guerreiro *et al*., 2016), lysozyme activity (Reda *et al*., 2013), natural hemagglutination activity (Ganguly *et al*., 2013), respiratory burst (Ibrahem *et al*., 2010), superoxide dismutase activity (Mona *et al*., 2015), and phagocytic activity (Ringø *et al*., 2010).

Compared with the control diet, FOS administration in Japanese flounder (*Paralichthys olivaceus*) significantly boosted lysozyme activity, except the phagocytic activity (Ye *et al*., 2011). The discrepancy in these findings has been reported possibly because of different prebiotics, level of inclusion, feed formulas and ingredients, fish species and their life stages, culture condition, duration and time of application.

FOS addition from 0.5 to 8 g kg^{-1} feed for two months significantly increased feed utilization, growth performances, and survival of the blunt snout bream, *M. amblycephala* (Wu *et al*., 2003). In contrast, the addition of FOS in diets (0.2–0.4% of dry weight) did not enhance the growth performance, immune modulation, and disease protection of large yellow croaker (Ai *et al*., 2011).

In this research, the significantly higher values of lysozyme and phagocytosis activities had observed since 30 days of FOS supplementary feeding. Akrami *et al.* (2013)

Table 3: Lysozyme activity of Climbing perch fed the diets with different levels of fructooligosaccharide (FOS) at two feeding levels, 3% of weight or satiation after 30–120 days

Feeding rate	FOS level (%)	Culture period			
		30 days	60 days	90 days	120 days
	0	10.05±0.15[a]	11.33±0.55[a]	10.21±0.54[a]	9.10±1.08[a]
3% of body weight	1	12.83±0.48[ab]	15.45±1.65[b]	16.03±1.41[c]	13.03±0.97[ab]
	2	11.86±1.16[ab]	16.06±1.45[b]	14.13±1.06[bc]	15.05±0.84[b]
	0	10.20±0.52[a]	11.25±0.20[a]	11.51±0.62[ab]	10.45±1.35[a]
Satiation	1	13.55±1.56[b]	18.38±0.74[b]	17.23±1.61[c]	15.76±1.86[b]
	2	14.38±0.82[b]	18.31±1.29[b]	13.98±0.35[bc]	14.88±1.59[b]
Two - way ANOVA					
Feeding rates		0.161	0.086	0.377	0.254
Prebiotic levels		0.009*	0.000*	0.001*	0.004*
interaction		0.435	0.393	0.746	0.569

Means ± SE with different superscripts in the same column are significantly different (P<0.05)
ns represents not significance (P > 0.05), while * is significant different (P < 0.05)

Table 4: Percent phagocytosis of Climbing perch fed the diets with different levels of fructooligosaccharide (FOS) at two feeding levels, 3% of weight or satiation after 30 – 120 days

Feeding rate	FOS level (%)	Culture period			
		30 days	60 days	90 days	120 days
	0	45.27±2.38[a]	43.89±1.99[a]	42.85±1.35[a]	41.31±3.18[a]
3% of body weight	1	57.29±2.83[b]	51.96±0.27[b]	50.35±1.91[b]	50.26±1.44[c]
	2	52.32±0.61[b]	51.96±1.48[b]	49.34±1.25[b]	49.79±1.33[c]
	0	42.50±0.80[a]	48.41±2.19[ab]	46.12±2.07[ab]	42.77±1.85[ab]
Satiation	1	52.68±0.57[b]	53.83±1.48[b]	49.93±1.91[b]	49.07±2.23[bc]
	2	45.19±3.12[a]	49.45±2.13[b]	48.01±1.96[ab]	47.65±1.81[abc]
Two - way ANOVA					
Feeding rates		0.013	0.377	0.734	0.718
Prebiotic levels		0.001*	0.006*	0.021*	0.006*
interaction		0.576	0.163	0.417	0.676

Means ± SE with different superscripts in the same column are significantly different (P<0.05)
ns represents not significance (P > 0.05), while * is significant different (P < 0.05)

reported that serum lysozyme activity enhanced significantly in a stellate sturgeon fed on the diet supplemented with 1% FOS compared with other treatments (P < 0.05); however, no significant effects were seen in serum lysozyme activity of Atlantic salmon fed with FOS compared with the control diet (Grisdale-Helland *et al*., 2008).

The advantageous outcomes obtained from previous researches inspire further studies on the feasible immune responses of various environmentally friendly feed additives. Additionally, the influences of the prebiotics on health should be investigated using fish challenged to bacterial infections or other stressors.

Conclusion

Supplementation of FOS had positive effects on growth performances and innate immune responses of Climbing Perch. Feeding 1% FOS additive diet to satiation was the most suited for Climbing Perch.

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