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Dietary fructooligosaccharide can mitigate the negative effects of immunity on Chinese mitten crab fed a high level of plant protein diet

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ABSTRACT

An 8-week feeding trial was carried out under controlled condition to evaluate the effect of dietary fructooligosaccharide (FOS) on growth performance, whole body composition, antioxidant status and immunity of crabs fed high levels of plant protein diets. Thus, six experimental diets were formulated (designated as F_0P_{50}, F_0P_{60}, F_0P_{70}, $F_{0.2}P_{50}$, $F_{0.2}P_{60}$ and $F_{0.2}P_{70}$), which contain two FOS levels (0 or 0.2%) and three plant protein levels (50, 60, or 70%) according to a 2 × 3 factorial design. The results showed that weight gain increased significantly as dietary plant protein level decreased from 70% to 50%. At 50% plant protein level, the addition of 0.2% FOS can significantly elevate weight gain (WG) ($P < 0.05$). The highest value in survival rate was observed in crabs fed $F_{0.2}P_{50}$ and $F_{0.2}P_{60}$ diet. Crabs fed $F_{0.2}P_{50}$ diet showed significantly higher crude protein content ($P < 0.05$) compared with those in other groups, but there were no significant differences in the contents of moisture, crude lipid and ash among all groups ($P > 0.05$). Catalase (CAT) activity in crabs fed $F_{0.2}P_{50}$ increased significantly ($P < 0.05$) compared with crabs fed F_0P_{60}, F_0P_{70}, $F_{0.2}P_{60}$ and $F_{0.2}P_{70}$, but malondialdehyde (MDA) concentrations decreased significantly ($P < 0.05$). Meanwhile, nitric oxide (NO) concentration, acid phosphatase (ACP) and alkaline phosphatase (AKP) activities of crabs fed 0.2% FOS diets increased significantly ($P < 0.05$) compared with crabs fed 0% FOS diets. The expressions of prophenoloxidase (*propo*) was significantly ($P < 0.05$) affected only by dietary plant protein levels with the highest values observed in 50% plant protein diet, whereas the opposite was true for Myeloid differentiation factor 88 (*myd88*). The mRNA expressions of mitochondrial manganese superoxide dismutase (*mtmnsod*), lipopolysaccharide-induced TNF-α factor (*litaf*) and toll like receptors (tlrs) were significantly affected ($P < 0.05$) by both FOS and plant protein levels. The cytosolic manganese superoxide dismutase (*cytmnsod*) mRNA expressions in $F_{0.2}P_{50}$ and $F_{0.2}P_{60}$ groups were significantly higher than those in F_0P_{70} and $F_{0.2}P_{70}$ groups. The results in this study indicated that supplementation with 0.2% FOS can enhance growth performance in crabs fed lower plant protein diets and as well improve immunity in those fed with higher plant protein diets.

1. Introduction

Chinese mitten crab (*Eriocheir sinensis*) is an economically important species in China and its farming may also be extended to other Asian countries. The worldwide fast-growing crab culture has resulted in an increase demand of pelletized feed year by year. And the crab is an omnivorous animal in natural environment, while the farmed crabs can be trained to eat artificial feeds. Most of the feed formulas of Chinese mitten crab contain fishmeal as the main protein sources. Feed has accounted for a large proportion of the total production cost [1]. Such large portion of cost is mainly due to high inclusion of expensive protein sources, especially fishmeal (FM).

Fishmeal has been the protein source of choice in aquafeed for many reasons, including its high protein content, excellent amino acid profile and high nutrient digestibility [2]. Nevertheless, the current use of fishmeal in the diets of fish and crustaceans is largely viewed as both an uneconomical and unsustainable practice [3]. Thus, the aquafeed industry had to search for alternative protein sources less expensive to reduce their dependence on FM [4,5]. Plant feedstuffs (PF) are nowadays the more available alternatives to FM, and can overcome the problems associated with the by-products of fish, such as organic and inorganic contaminants, shortage of supply and net effect of demand-

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and-supply economics [6]. However, the negative effects on plant feedstuffs, such as anti-nutritional factors, imbalance of amino acids and less palatability, and species differences should be the major factors for this wide variation in the replacement levels [7]. An alternative approach to reduce adverse factors of plant protein may be the inclusion of diet additives that can improve growth performance in aquatic and, possibly, compensate performance loss in low FM diets [8].

A range of diet supplements, including prebiotics and probiotics were commercially available for aquatic animals. Prebiotics appear as an alternative that similarly allow to improve fish health status by modulating the host gut microbiota. Prebiotics has shown promise as eco-friendly additives to improve growth performance, feed utilization, immunological status and disease resistance in aquaculture [9–12]. Fructooligosaccharides (FOS), mannanoligosaccharides (MOX), galactooligosaccharides (GOX), xylooligosaccharide (XOS) and other related carbohydrates have all received considerable attention due to their health benefits to fish [12]. For instance, FOS supplementation positively affected growth performance, survival and feed utilization of the blunt snout bream (*Megalobrama amblycephala*) [13]. Soleimani et al. reported that 2% and 3% FOS can be considered as a beneficial dietary supplement for improving the immune response, stress resistance and digestive enzyme activities of Caspian roach (*Rutilus rutilus*) fry [14]. Li et al. reported that FOS supplementation influenced potentially on growth performance, feed utilization, intestinal microflora and immunity of Pacific white shrimp (*Litopenaeus vannamei*) [15]. In addition, Guerreiro studied that plant feedstuffs supplemented with FOS can decrease the activity of superoxide dismutase (SOD), but scFOS had little effect of oxidative status on European sea bass (*Dicentrarchus labrax*) [6]. However, up to date, there was no report concerning plant feedstuffs supplemented with FOS on growth, immunity and disease resistant in crabs and therefore, further studies were required.

Thus, this study was particularly relevant when FM was highly replaced by PF and to evaluate the effects of dietary FOS on the growth performance, whole body composition, antioxidant status and immune of crabs. The present work will elucidate the potential of prebiotics to mitigate some of the negative effects on crab's health as feeding PF-based diets, which will contribute to result in functional diets with both health and environmental benefits.

2. Materials and methods

2.1. Experimental diets

The FOS product was obtained from by Meiji Holdings Co., Ltd., Japan and characterized as having a typical composition of 95% 1-3fructose. A 2 (FOS level) × 3 (Plant protein level) factorial design with three replicates per dietary treatment was adopted in this study. So, six experimental diets (designated as F_0P_{50}, F_0P_{60}, F_0P_{70}, $F_{0.2}P_{50}$, $F_{0.2}P_{60}$ and $F_{0.2}P_{70}$), which contained 0 or 0.2% dietary FOS combined with plant protein at 50, 60, or 70%. The diets were prepared by sieving the protein sources, and all dry ingredients were thoroughly mixed, followed by the addition of the lipid sources, and thoroughly mixing again. Distilled water was then added at 30% of the ingredient weight and after mixing for 15 min, the resulting mash was then pelleted through a 2.5 mm diameter die and extruded through a single-screw meat grinder extruder. Extruded diets were dried in the ventilation room for 24 h, ground to appropriate size for crabs, and then stored in sealed plastic bags at −20 °C until used. Proximate analyses of the experimental diets (Table 1) were determined according to the method of AOAC [16]. Crude protein content was determined by Kjeldahl method using an Auto Kjeldahl System (KjeltecTM 2300, Foss, Sweden). Crude lipid was analyzed by Soxtec system, moisture content by a dry oven (D-63450, Heraeus, Hanau, Germany) drying at 105 °C for 24 h and ash by a furnace muffler (550 °C for 4 h). Crabs were hand-fed twice daily at 08:00 a.m. and 18:00 p.m. for 8 weeks.

Table 1
Ingredients and proximal composition of experimental diets.

	Experimental diets					
	F_0P_{50}	F_0P_{60}	F_0P_{70}	$F_{0.2}P_{50}$	$F_{0.2}P_{60}$	$F_{0.2}P_{70}$
Ingredients (% dry matter)						
Fish meal	27.58	22.8	17.55	27.58	22.8	17.55
Soybean meal	22.31	26.33	30.45	22.31	26.33	30.45
Peanut meal	14.55	17.17	19.89	14.55	17.17	19.89
Rapeseed meal	4.85	5.72	6.68	4.85	5.72	6.68
Wheat flour	19	16.27	13.72	19	16.27	13.72
Soybean oil	1.46	1.46	1.46	1.46	1.46	1.46
a-Cellulose	0.2	0.2	0.2	0	0	0
FOS	0	0	0	0.2	0.2	0.2
Fish oil	0.97	0.97	0.97	0.97	0.97	0.97
$Ca(H_2PO_4)_2$	2.16	2.16	2.16	2.16	2.16	2.16
Attapulgite clay	0.97	0.97	0.97	0.97	0.97	0.97
Zeolite powder	0.97	0.97	0.97	0.97	0.97	0.97
Blood powder	1.94	1.94	1.94	1.94	1.94	1.94
Premix[a]	1.00	1.00	1.00	1.00	1.00	1.00
Mixture[b]	2.04	2.04	2.04	2.04	2.04	2.04
Proximate analysis (% dry weight)						
Dry matter	90.28	89.35	91.54	90.82	89.49	89.23
Crude protein	40.86	40.70	39.95	39.96	40.23	39.63
Crude lipids	5.24	5.35	5.35	5.45	5.51	5.46
Ash Starch	13.68	13.83	13.94	13.83	13.89	13.85

Note:[a] Premix supplied the following minerals (g/kg) and vitamins (IU or mg/kg): $CuSO_4{\cdot}5H_2O$, 2 g; $FeSO_4{\cdot}7H_2O$, 25 g; $ZnSO_4{\cdot}7H_2O$, 22 g; $MnSO_4{\cdot}4H_2O$, 7 g; Na_2SeO_3, 0.04 g; KI, 0.026 g; $CoCl_2{\cdot}6H_2O$, 0.1 g; Vitamin A, 900,000 IU; Vitamin D, 200,000 IU; Vitamin E, 4500 mg; Vitamin K_3, 220 mg; Vitamin B_1, 320 mg; Vitamin B_2, 1090 mg; Vitamin B_5, 2000 mg; Vitamin B_6, 500 mg; Vitamin B_{12}, 1.6 mg; Vitamin C, 10,000 mg; Pantothenate, 1000 mg; Folic acid, 165 mg; Choline, 60,000 mg; Biotin, 100 mg; Myoinositol 15,000 mg.[b] Mixture includes the following ingredients (%): choline chloride 4.75%; antioxidants 1.72%; mildew-proof agent 2.35%; salt 22.06%; Lvkangyuan 59.30% and biostimep 9.51%.

2.2. Crab and experimental design

Chinese mitten crab were collected from a local farm in Pukou, Jiangsu province, China. Crabs were fed a commercial diet (Haipurui Feed Co., Ltd., Jiangsu) twice a day and acclimated to experimental conditions for 2 weeks. A total of 288 crabs (average initial weight: 30.85 ± 1.02 g) were randomly distributed into 18 cement pools (2.0 × 2.0 × 0.5 m, L: W: H) at a density of 16 crabs per pool. During the experimental period, water temperature, pH and dissolved oxygen were monitored daily and recorded as 24 ± 2 °C, 8.5–8.6 and 5 mg/L respectively.

2.3. Sample collection

Crabs were anaesthetized on ice for 10 min. Because of low temperature can reduce the crab vigor. After that, six crabs were sampled in each pool. Hemolymph was collected using syringes from each crab's second last pair of walking legs, mixing 1:1 with precooling anticoagulant solution (100 mmol L− 1 glucose, 26 mmol L− 1 citrate, 30 mmol L− 1 citric acid, 450 mmol L− 1 NaCl, 10 mmol L− 1 EDTA, pH = 7.2) [17] and then immediately centrifuged at 9000 rpm and 4 °C for 20 min. After that, hepatopancreas was dissected aseptically and frozen immediately in liquid nitrogen and stored at −80 °C for subsequent analysis.

2.4. Growth performance

At the end of feeding trial, all crabs were deprived of food for 24 h before weighing and sampling, and then the following parameters were measured:

Weight gain (WG) = $100 \times (W_2 - W_1) / W_1$