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Fishmeal replacement by rice protein concentrate with xylooligosaccharides supplement benefits the growth performance, antioxidant capability and immune responses against *Aeromonas hydrophila* in blunt snout bream (*Megalobrama amblycephala*)

Kenneth Prudence Abasubong, Wen-Bin Liu, Ding-Dong Zhang, Xiang-Yang Yuan, Si-Lei Xia, Chao Xu, Xiang-Fei Li*

Key Laboratory of Aquatic Nutrition and Feed Science of Jiangsu Province, College of Animal Science and Technology, Nanjing Agricultural University, No. 1 Weigang Road, Nanjing, 210095, People's Republic of China

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ABSTRACT

This study aimed to investigate the effects of fishmeal (FM) replacement by rice protein concentrate (RPC) with a xylooligosaccharides supplement on the growth performance, antioxidant capability and immune response against *Aeromonas hydrophila* in blunt snout bream (*Megalobrama amblycephala*). Fish (46.85 ± 0.34 g) were randomly assigned to one of 6 diets, namely the control diet (containing FM), the RPC diet (FM replaced by RPC) and RPC diet supplemented with 0.5, 1.5, 2.3 and 3% XOS respectively, for 8 weeks. After the feeding trial, fish were challenged by *Aeromonas hydrophila* for 96 h with the blood and liver sample obtained at 48 and 96 h respectively. The results showed that the final weight, weight gain and protein efficiency ratio of fish fed RPC diet were significantly ($P < 0.05$) lower than that of the control group, whereas the opposite was true for FCR. However, the supplement of 1.5% XOS remarkably ($P < 0.05$) improved these parameters compared to the control diet. Plasma total iron binding capacity of fish fed the RPC diet showed little difference ($P > 0.05$) with that of the control group, but it enhanced significantly ($P < 0.05$) with the supplement of 1.5% XOS compared to the control group. After bacterial infection, plasma lysozyme (LYM), complement 3, complement 4, myeloperoxidase (MPO), acid phosphatase (ACP) and alkaline phosphatase activities, as well as immunoglobulin M, levels all increased significantly ($P < 0.05$) with the maximum value is attained at 48 h, then they decreased significantly ($P < 0.05$) with further increasing time at 96 h. Similar results were also observed in liver superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activities as well as malondialdehyde (MDA) content. Regarding dietary treatment, these parameters of fish fed RPC diet showed little difference ($P > 0.05$) with those fed the control diet but were significantly enhanced ($P < 0.05$) when RPC diet was supplemented with 1.5–2.3% XOS, except for hepatic (MDA) content which showed an opposite trend compared to the control group. After 96 h of challenge, the relative percentage survival (RPS) of fish fed XOS was significantly higher ($P < 0.05$) than that of fish offered the control and RPC diet. In addition, significant ($P < 0.05$) interactions between dietary XOS and sampling time were also observed in plasma LYM, MPO, ACP, and hepatic SOD, CAT, GPX, MDA, as well as RPS. In conclusion, this study indicated that dietary supplementation of 1.5%XOS could significantly improve the growth performance, antioxidant capability, innate immunity and *A. hydrophila* resistance of blunt snout bream fed diets with FM replaced by RPC.

1. Introduction

Fish meal (FM) has usually been the primary source of protein in practical fish diets due to its high level of protein, high digestibility, and few anti-nutritional factors [1]. However, as a result of the increase in aquaculture activity in recent years, the availability of FM is now limited [2]. Thus, to allow the sustainable development of this industry, it is of great significance to find other protein sources to replace FM in fish diets. In this regard, plant protein (PP) meals are good candidates due to their rich abundance and relatively low cost [3]. Recently, rice protein concentrate (RPC) has become commercially available for use in the animal and fish feed industry [4,5]. RPC has a high nutrient

* Corresponding author.
E-mail address: xfli@njau.edu.cn (X.-F. Li).

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content, i.e., around 66–70% of protein and 10–11% of lipid [5], making it a perfect replacer of FM. Unfortunately, like other PP meals, the deficiency of lysine limits its utilization in aquafeeds [5,6]. Also, PP sources including RPC are generally rich in anti-nutritional factors, which have been proven to cause poor palatability and low digestibility of feed as well as the growth retardation and compromised immunity of fish [7]. Also, PP-based diets could enhance the hepatic lipid peroxidation levels of fish compared with that received FM-based diets, as was coupled with the poor health status [8]. Considering this, it is quite necessary to find a practical and practical approach to improve the growth performance, antioxidant capability and innate immunity of fish offered PP-based diets.

Prebiotics has been defined as “non-digestible food ingredients, which can beneficially affect the host by selectively stimulating the growth and activity of health-promoting bacteria in the intestinal tract [9]. Through this, they could modulate the immune status, decrease the oxidative damage or increase the antioxidant potential, and improve the gut morphology of the host [10,11]. These improvements generally can be achieved for instance, directly through the interaction with pattern recognition receptors, or indirectly through an increase in specific bacteria groups and the by-products produced as a result of prebiotic fermentation [11,12]. According to published literature, prebiotics such as mannooligosaccharides (MOS), fructooligosaccharides (FOS) and, inulin has shown promise as environmentally friendly preventives in aquaculture, especially for fishes [13–15]. For instance, the incorporation of FOS into PP-based diets could enhance the immunity and affect reactive oxygen species generation in fish, decreasing oxidative damage and increasing antioxidant potential [16]. Also, inulin has been reported enhancing the antioxidant capacity of fish fed PP-based diets with ROS scavenging ability [17]. However, such information is still quite limited in xylooligosaccharides(XOS), which attracts much interest recently. XOS is a product of the hydrolysis of xylan, which in turn is an essential component of hemicellulose [18]. To date, XOS has been demonstrated to exhibit some health benefits on fish, such as the enhancement of antioxidant capacity and the anti-inflammatory and anti-microbial functions [18,19]. For instance, an enhancing effect on growth performance and digestive enzyme activities was observed in Crucian carp (*Carassius auratus gibelio*) fed an XOS-supplemented diet [20]. The mucus bactericidal activity and plasma total protein content both increased in Caspian white fish *Rutilus frisii kutum* given diets supplemented with 3% XOS [21]. Also, an improvement of the immune status was observed in white sea bream *Diplodus sargus* upon XOS administration, as the alternative complement pathway and lysozyme activities as well as total plasma immunoglobulin levels were increased [22]. However, the afore-mentioned studies mainly focused on the XOS supplementation in FM-based diets. Whether XOS could improve the growth performance and innate immunity of fish fed diets with FM entirely replaced by PP meals is still unknown.

Blunt snout bream *Megalobrama amblycephala* is an attractive herbivores fish, which has been extensively cultured in Asia for a long time because of its high larval survival rate, fast growth, tender flesh, high diseases resistance and the use of natural foods [23]. Recently, the culture of this species has increased remarkably due to its high nutritional value coupled with the favorable economic returns. To maximize the culture profit, diets formulated for this species usually contain a significant proportion of PP in China. However, this cultural practice has inevitably led to the poor growth, compromised disease resistance and high mortality of this species, especially during summer months, where high temperatures seem to trigger disease outbreak caused by *Aeromonas hydrophila* [14]. *Aeromonas hydrophilia* are among the most common bacteria in freshwater habitats throughout the world [24]. They are the prominent microbiota in freshwater reservoirs where they are together with other microorganisms, act as natural bio-filters and promote self-purification of water [25]. However, this bacteria frequently cause infections and diseases of fish [24], as consequently results in substantial economic losses [26]. Therefore, it is quite urgent to promote the PP utilization of this species by developing effective strategies to avoid growth retardation and immunosuppression. With all this in mind, this study was conducted to evaluate the effects of XOS supplementation on the growth performance, antioxidant capability and immune responses against *Aeromonas hydrophila* in blunt snout bream fed diets with FM completely replaced by RPC.

2. Material and method

2.1. Diet formulations

XOS used in the present study was provided by ShanghaiYuanye biotechnology co., ltd china. According to the manufacturer, it was 99% pure. A total of six diets were formulated, namely, the control diet (containing FM), the RPC diet (FM entirely replaced by RPC) and RPC diets supplemented with 0.5%, 1.5%, 2.3% and 3% XOS, respectively. All ingredients were weighed and mixed using a laboratory feed mixer. After that, a certain amount of water was later added to form a soft dough. The dough was then pelleted using a pellet machine. The experimental feed was dried at room temperature and stored in separate plastic containers at 4 °C until used. The chemical composition of main protein sources (FM and RPC) is given in Table 1, while the formulation and proximate composition of the experimental diets are presented in Table 2. The amino acids composition of the experimental diets was shown in Table 3.

2.2. Experimental fish and feeding trial

Blunt snout bream were obtained from a local fish hatchery (Nanjing, China). Before the feeding trial, fish were acclimated to experimental conditions for two weeks. During the acclimation period, fish were fed a control diet three times a day. After that, 480 healthy fish with an initial mean body weight of 46.85 ± 0.34 g were randomly distributed into 24 floating net cages (1 × 1 × 1 m, Length: Width: Height), which were anchored in an outdoor pond (100 × 40 m, Length: Width). Each cage held 20 fish. Then, fish were randomly assigned to one of six diets. Each treatment has four replicates. Fish were fed three times daily at 7:00, 12:00 and 17:00 h respectively for eight weeks with feed consumption recorded. Fish were hand-fed to apparent satiation with utmost care to minimize feed waste. Fish were held under

Table 1
Proximate compositions and amino acid compositions of the main protein sources used in the diet.

Composition (%, dry matter)	FM	RPC
Crude protein	66.40	66.00
Crude lipid	7.04	7.06
Essential amino acids (%)		
Threonine	2.62	2.56
Valine	3.01	3.51
Methionine	1.95	2.00
Isoleucine	2.61	2.57
Leucine	4.74	5.33
Phenylalanine	2.94	3.71
Lysine	4.99	1.78
Histidine	1.65	1.45
Arginine	3.00	4.27
Non-Essential amino acids (%)		
Aspartic acid	5.60	5.80
Serine	2.54	3.15
Glutamic acid	7.77	10.73
Glycine	3.73	2.80
Alanine	3.72	3.65
Cysteine	0.60	1.37
Tyrosine	2.18	3.24
Proline	2.36	2.09

FM, fishmeal; RPC, rice protein concentrate.