Fish & Shellfish Immunology 41 (2014) 560–569

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

# Effects of dietary fructooligosaccharide levels and feeding modes on growth, immune responses, antioxidant capability and disease resistance of blunt snout bream (*Megalobrama amblycephala*)

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ARTICLE INFO

*Article history:*
Received 20 June 2014
Received in revised form
3 October 2014
Accepted 3 October 2014
Available online 16 October 2014

*Keywords:*
*Megalobrama amblycephala*
Fructooligosaccharide
Feeding modes
Immunity
Disease resistance

ABSTRACT

This study aimed to determine the effects of fructooligosaccharide (FOS) levels and its feeding modes on growth, immune response, antioxidant capability and disease resistance of blunt snout bream (*Megalobrama amblycephala*). Fish (12.5 ± 0.5 g) were subjected to three FOS levels (0, 0.4% and 0.8%) and two feeding modes (supplementing FOS continuously and supplementing FOS two days interval 5 days) according to a 3 × 2 factorial design. At the end of 8-week feeding trial, fish were challenged by *Aeromonas hydrophila* with concentration of $1 \times 10^5$ CFU $mL^{-1}$ and mortality was recorded for the next 96 h. Fish fed 0.4% FOS continuously (D2) and fish fed the basal diet for 5 days followed by 0.8% FOS for 2 days (D5) showed admirable growth performance. The highest plasma lysozyme, acid phosphatase and myeloperoxidase activities as well as complement component 3, total protein and immunoglobulin M (IgM) levels were all observed in fish fed D5. They were significantly higher ($P < 0.05$) than those of the control group and/or fish fed 0.8% FOS continuously, but exhibited no statistical difference ($P > 0.05$) with that of fish fed D2. A similar trend was also observed in antioxidant capability as well as the expression of Leap-I and Leap-II. Mortality showed an opposite trend with the immune response with the lowest rate observed in fish fed D5. The results indicated that diet supplementing FOS in appropriate levels and feeding modes could improve the growth, immune response and antioxidant capability of fish, as might consequently lead to enhanced disease resistance. It can be speculated that the basal diet for 5 days followed by 0.8% FOS for 2 days was most suitable for blunt snout bream.



## 1. Introduction

Blunt snout bream (*Megalobrama amblycephala*) is one of the most important species cultured in China. This fish is suitable for intensive aquaculture because of its fast growth, ease of reproduction, good taste and high market value [1]. However, the rapid expansion of the production has resulted in the emergence of several resistant pathogens, which is highly infectious and lethal to this species. Thus, improving and protecting fish health in commercial production practices is a major factor in the aquaculture industry. One of the most common ways to enhance fish immunity is to administer antibiotics. However, the adverse effects are notorious, which includes the development of antibiotic resistance of aquatic microorganisms and the accumulation of antimicrobial residues in products [2]. Therefore, alternative strategies such as the application of vaccine, probiotics, prebiotics, and immunostimulants may help to reduce the susceptibility of fish to diseases. According to published literatures, the prebiotics such as mannanoligosaccharides (MOS), fructooligosaccharides (FOS), inulin and vitamin C has shown promise as preventive and environmentally friendly alternatives to antibiotics in aquaculture, especially for fishes [3–7].In fact, during the last decade the application of prebiotics has been increasing in aquaculture.

Among prebiotics, fructooligosaccharides (FOS) is nondigestible carbohydrates, which selectively stimulate the growth and metabolism of health-promoting bacteria present in the host gut. It is reported that FOS could overcome the limitations and side effects of

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http://dx.doi.org/10.1016/j.fsi.2014.10.005
1050-4648/

antibiotics and other drugs, leading to high production through enhanced growth, stimulated immune response and increased resistance to pathogens of fish [3,5,8–11]. Our previous studies also demonstrated the efficacy of FOS to increase the growth performance and the non-specific immunity of fish [8,11]. In previous studies, FOS was supplemented continuously. However, some researchers have proved that continuous administration of high levels of immunostimulants has no beneficial effects on growth or/and immunity [12,13]. Others also suggested that discontinuous administration of immunostimulants may solve those problems [12]. Unfortunately, up to date, there is no report concerning the feeding modes of FOS on growth, immunity and disease resistant in fish.

Being the important components of innate immunity systems antimicrobial peptides (AMPs) are now widely acknowledged as key marker molecules for the assessment of the efficacy of immunostimulants [14]. Since the aquatic environment provides considerable exposure to various pathogens, fish possess a very large number of AMPs against a broad spectrum of pathogens [15]. Among these, liver expressed antimicrobial peptides (Leap) have drawn considerable attention due to its important role in innate immune defense [16]. So far, two categories of Leap (namely Leap-1 and Leap-2) have been identified [17]. However, there is little information concerning the Leap expression following immunostimulants treatment. The correlation between Leap expression and fish immunity still remains poorly understood.

Bearing these in mind, this study was conducted to assess the effect of dietary FOS and its feeding modes on growth, innate immune responses and disease resistance to *Aeromonas hydrophila* in blunt snout bream The data obtained here may give some instructions for the application of prebiotics in herbivorous freshwater fish.

## 2. Materials and methods

### 2.1. FOS and experimental diets

The FOS used in this study was produced by Meiji Holdings Co., Ltd, Japan. The minimum level of sucrose combined with 1–3 fructoses in the product was 95% and the level of other components was no more than 5%, mainly including glucose, fructose and sucroser.

The composition of the basal diet is shown in Table 1. Fish meal, soybean meal, cottonseed meal and rapeseed meal served as protein sources. Both fish oil and soybean oil (1:1) were used as lipid sources. Wheat flour was adopted as carbohydrate sources. Graded doses of FOS (0, 0.4% and 0.8%) were added into the basal diet followed by mixing manually. Dietary ingredients were ground into fine powder then thoroughly mixed, and then blended with an additional 100 mL of water per kg of diet to form a soft dough which was pelleted (without injected steam) using a Pillet Mill with a 2 mm diameter die. The experiment feed was dried at air temperature at 33 °C overnight and stored in sealed plastic bags at −4 °C until use. The proximate composition of the experimental diets was determined according to the standard AOAC methodology [18].

**Table 1**
Ingredients and proximate composition of the basal diet.

| Ingredients (g $kg^{-1}$) | | Proximate composition (g $kg^{-1}$air-dry basis) | |
|---|---|---|---|
| Fish meal | 80 | Moisture | 114.4 |
| Soybean meal | 300 | Crude protein | 327.1 |
| Cottonseed meal | 150 | Crude lipid | 68.8 |
| Rapeseed meal | 150 | Energy (MJ $kg^{-1}$) | 15.1 |
| Soybean oil | 22 | | |
| Fish oil | 22 | | |
| Wheat bran | 50 | | |
| Wheat flour | 196 | | |
| $Ca(H_2PO_4)_2$ | 18 | | |
| Premix[a] | 10 | | |
| Salt | 2 | | |

[a] Premix supplied the following minerals (g $kg^{-1}$) and vitamins (IU or mg $kg^{-1}$): $CuSO_4 \cdot 5H_2O$, 2.0 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, 5000 mg; Pantothenate, 1000 mg; Folic acid, 165 mg; Choline, 60,000 mg.

### 2.2. Fish and experimental design

Blunt snout bream were obtained from a local fish hatchery (Nanjing, China). Prior to the feeding trial, fish were acclimated to experimental conditions for 4 weeks. During the acclimation period, fish were fed a control diet three times a day. And then, 600 healthy fish with an initial mean body weight of 12.5 ± 0.5 g were randomly distributed into 20 cages which were anchored in an outdoor pond. Each treatment has four replicates and each cage (1 × 1 × 1 m, L:W:H) holds 30 fish. A total of five treatments were adopted. Control group was fed with the basal diet (Diet 1, D1). The second group was fed with the basal diet supplemented with 0.4% FOS (Diet 2, D2) continuously. The third was fed with the basal diet supplemented with 0.8% FOS (Diet 3, D3) continuously. The fourth was fed with the basal diet for 5 days followed by 0.4% FOS for 2 days (Diet 4, D4), and the fifth was fed with the basal diet for 5 days followed by 0.8% FOS for 2 days (Diet 5, D5). Fish were fed three times daily at 7:00, 12:00 and 17:00 h, respectively, for 8 weeks. Fish were hand-fed to apparent satiation with utmost care to minimize feed waste. Fish were held under natural photoperiod throughout the feeding trail. Water temperature, pH and dissolved oxygen were monitored using a YSI 556 MPS multi-probe field meter (Geotech, USA). Water temperature ranged from 23 to 28 °C, pH fluctuated between 6.5 and 7.6 and dissolved oxygen was maintained approximately at 5.0 mg $L^{-1}$ during the feeding trial.

### 2.3. Sampling and analysis

#### 2.3.1. Sampling

At the end of the feeding trial, fish were starved for 24 h before sampling. And then all individuals were quickly removed from each cage and anesthetized in diluted MS-222 (tricaine methanesulfonate, Sigma, USA) at the concentration of 100 mg $L^{-1}$. Total number and weight of fish in each cage were determined to calculate the growth performance. Six fish were randomly removed from each cage and blood sample was collected by caudal vein puncture using heparinized syringes coated with lithium heparin as anticoagulant. After centrifugation (3000 g for 10 min at 4 °C), plasma was stored at −80 °C for subsequent analysis. In addition, individual liver and intestinal were dissected over an ice bed and washed thoroughly with chilled saline (0.89 g NaCl $L^{-1}$), dried quickly over a piece of filter paper and stored at −80 °C. Three livers and intestines were used for enzymatic analysis and another three livers were used for Real-time PCR.

#### 2.3.2. Growth performance

The fish were weighed individually before (initial body weight) and after (final body weight) the 56-day feeding experiment. For each treatment, all fish were used to quantify the percent of weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR). These parameters were calculated as follows:

$$\text{Weight gain (WG)} = 100 \times (W_f - W_i)/W_i$$