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Dietary β-glucan improved growth performance, *Vibrio* counts, haematological parameters and stress resistance of pompano fish, *Trachinotus ovatus* Linnaeus, 1758

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A B S T R A C T

This study evaluated effects of graded levels of dietary β-glucan (0, 0.5, 1, 2 and 4 g kg^{-1}) on growth performance, haematological parameters, intestinal *Vibrio* counts, dose requirement and salinity stress resistance in pompano fish *Trachinotus ovatus* (6.45 g ± 0.06 (SEM)). After 8-weeks of diet feeding, growth was significantly higher in fish fed diets with 0.10% β-glucan compared to fish fed control diet (no β-glucan). Survival increased significantly in fish fed 0.05 and 0.10% β-glucan compared to control diet. There were significant increases in red blood cells (in 0.20% β-glucan diet), in total leukocytes (in 0.05–0.20% β-glucan diet), in both lymphocyte and monocyte count in fish fed 0.10%–0.40% β-glucan diet. However, dietary β-glucan did not affect neutrophil, eosinophil and basophil counts. Intestinal *Vibrio* counts were reduced in fish fed any level of β-glucan compared to control. In addition, dietary β-glucan levels highly correlated with growth, survival, intestinal *Vibrio* counts and haematological index. Optimal β-glucan levels for maximal growth of fish were predicted to be 0.122% at day 21 (R^2 = 98.53%), 0.120% at day 28 (R^2 = 78.55%), 0.115% at day 42 (R^2 = 62.21%) and 0.090% at day 56 (R^2 = 75.18%), showing a decreasing β-glucan requirement with increasing fish size. Furthermore, optimal β-glucan levels for maximal haematological parameters based on lymphocyte count, was estimated to be 0.120% (R^2 = 98.53%) at day 56. Also, fish fed 0.05%–0.20% β-glucan showed better resistance against salinity stress. In conclusion, β-glucan supplementation is effective for improving growth, intestinal *Vibrio* counts and boosted stress resistance of the pompano fish, *T. ovatus*.

1. Introduction

Aquaculture is one of the fastest developing sectors in the global food industry with a growth of 6.2% between 2000 and 2012 [1]. For many decades, antibiotics have been used as a growth promoter and disease prevention tool in aquaculture [2]. However, antibiotics cause considerable risks to the aquatic species, the environment and to humans [3]. Therefore, there has been increased research on alternatives to antibiotic additives. Besides vaccine development, probiotics, prebiotics and immunostimulants have received increasing consideration.

Glucan-specific receptors are present on phagocytic cell membranes of several species [4]. It was reported that β-glucan administration improved immunological responses of the host, intestinal microbiota and disease resistance in invertebrates [5,6] as well as improving growth of many aquaculture species [7]. In aquaculture, β-glucan administration was reported to improve growth performance among species including koi carp (*Cyprinus carpio koi*) [8], large yellow croaker (*Pseudosciaena crocea*) [9], rohu (*Labeo rohita*) [10] and snapper (*Pagrus auratus*) [11]. However, other studies did not show positive effects on growth performance, e.g. European sea bass (*Dicentrarchus labrax*) [12]. In addition to growth enhancement, β-glucan had been recognised to enhance immune functions of many aquaculture species including Atlantic salmon (*Salmo salar)* [13], Indian major carp (*Catla catla*) [14] and tench (*Tinca tinca*) [15] or to increase disease resistance in carp upon challenges with *Edwardsiella tarda* [16], zebrafish challenged with *Aeromonas hydrophila* [17] and Atlantic salmon challenged with *Edwardsiella ictaluri* [18]. Although there have been many studies on the effects of β-glucan on aquaculture species, to the authors' knowledge, effects on pompano fish have not been studied previously.

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Pompano fish, *Trachinotus ovatus* belonging to the family Carangidae, is a high value aquaculture species which is popularly cultured in Asia [19]. However, pompano aquaculture production is occasionally impacted by diseases. At least five bacterial species were found from diseased snubnose pompano, *Trachinotus blochii*, including *Streptococcus* spp., *Escherichia coli*, *Salmonella* spp., *Pseudomonas* spp. and *Vibrio* spp. [20]. Nocardiosis disease caused by *Nocardia seriolae* bacteria, characterised by nodules in gills, kidney and spleen of *Trachinotus* has been observed. This disease is of increasing concern with increased mortality in pompano fish (*Trachinotus*) [21]. Furthermore, in aquaculture, fish are also affected by handling, transport, air exposure and environmental fluctuation including salinity which cause negative effects on growth performance, immune response and mortality [22,23].

Although there are many studies on effects of β-glucan on aquatic species, information on the effects of dietary β-glucan on pompano fish is scarce. No published study has determined optimal inclusion levels, particularly on animal size and time of administration. This is important as an inappropriate dosage of the prebiotic supplement may adversely affect the animals. It has been reported that micronutrient inclusion at a higher dosage has caused negative effects on growth [24,25]. In addition, there is no study on the effects of dietary β-glucan supplement on physical stress resistance such as air exposure of pompano fish.

The aim of the present study is to examine the effect of graded levels of dietary β-glucan on growth performance, haematological parameters, gut *Vibrio* counts and salinity stress tolerance for pompano *T. ovatus*.

2. Materials and methods

2.1. Experimental fish and culture condition

Pompano juveniles, *Trachinotus ovatus* (6.45 g ± 0.06, mean weight ± SEM) were obtained from a local hatchery, transported to Institute of Oceanography, Nha Trang, Vietnam, and acclimated for 2 weeks. During the acclimation, the fish were fed commercial diet (INVE for fish) at 5% of their body weight and observed satiation. Each culture tank (300 L, sized 50 × 80 × 80 cm) was equipped with an independent recirculation system with a flow rate at ~500 L per hour. Fish were kept under natural photoperiod condition throughout the feeding trial. Water parameters were temperature: 28.5–29.0 °C; NH_4/NH_3: <0.05 mg L^{-1}; NO_2: <0.01 mg L^{-1}, salinity: 34–35 ppt and pH: 8.0–8.4.

2.2. Diet preparation

In order to limit the effect of β-glucan existing in natural feed ingredients, the basal diet was formulated as per a previous dietary β-glucan administration on fish [10] with some modification for nutritionally adequacy of pompano fish, *T. ovatus* [19]. The basal diet (D0.0) was then supplemented with β-glucan (Marcrogard®, Biorigin, Brazil) to give 0.05% (D0.05), 0.10% (D0.10), 0.20% (D0.20) and 0.40% (D0.40) of β-glucan in the diets. Diets were cold extruded into 2 mm pellets, air-dried and then stored in plastic bags at 4 °C until use. The composition of the basal diet is listed in Table 1.

Table 1
Composition of the basal diet with crude protein 48.6%, crude lipid 6.7%, gross energy 19.78 MJ kg^{-1}, dry matter 92.1% and ash 11.31%.

Ingredients	Percentage
Fish meal (62% - Vietnam)	44.60
Gluten (wheat)	21.10
Soybean	10.40
Fish oil	3.60
Binder	1.10
Mineral premix	1.50
Vitamin premix	1.50
Corn starch	16.20
TOTAL	**100**

2.3. Experimental design

The experiment was designed to compare growth and mortality of fish fed on the basal diet supplemented with four graded concentrations of β-glucan as detailed in Section 2.2. After acclimation, twenty-four fish were randomly stocked in each of six replicate tanks designed for each diet treatment group and fed for 56 days. During the experimental period, fish were hand-fed to apparent satiation twice daily (8:30 and 17:00 h).

2.4. Sampling and data collection

During the feeding trial, all fish were weighed and total length was measured at the beginning (day 0) and again every 14 days. Fish were starved for 24 h prior to weighing and sampling. Number of dead fish was recorded daily. At day 56 (end of feeding trial), one fish from each replicate tank (6 fish per treatment) was randomly sampled and immediately euthanized by 100 mg L^{-1} of MS-222 (tricaine methanesulfonate; Sigma, USA). Weight and length were also measured prior to blood sampling. Blood samples were withdrawn from the caudal peduncle by using 1 mL syringe with 21 gauge needles as soon as the fish lost equilibrium and blood was then quickly transferred to heparinised tubes for total erythrocyte and leukocyte counts. Mid intestine was collected and stored in ice for determining *Vibrio* bacteria within the day.

2.5. Haematological assays

The red blood cells (RBC) and white blood cells (WBC) were counted using haemocytometer [26]. Differential leukocyte counts (lymphocytes, monocytes, neutrophils, basophil and eosinophils) were obtained by blood smear preparations, air-dried, fixed in methanol, and stained using Diff-Quick® (Medion Diagnostics GmbH, Düdingen, Germany). Two hundred leukocytes in each blood smear were categorized based on their morphology under a microscope at 1000× magnification.

2.6. Intestinal Vibrio isolation

For the evaluation of the intestinal microbial communities, mid intestine samples from six fish in each treatment were taken at day 56. Fish were cleaned in 2% sterilized saline water. The gut was dissected and ground by hand using a ceramic mortar and pestle after quick alcohol fire sterilization. The ground tissue was mixed with 0.85% saline water at a ratio of 1:10, and analysis for *Vibrio* spp. was performed using thiosulfate citrate bile salt sucrose (TCBS) agar according to APHA standard method [27]. The culture plates were incubated at 37 °C for 24 h, total colony-forming units (cfu) were recorded and then calculated per unit of tissue (g).

2.7. Predicting β-glucan requirement for maximal growth and immune responses of pompano fish

In the present study, as weight gain (WG, g d^{-1}) (representing growth), and haematological lymphocyte count (representing immune function), showed the highest statistically significant correlation with the dietary β-glucan concentrations, they were used to predict the concentration requirement for maximal growth and immune response of pompano fish at each collection time using