Effects of dietary β-1,3/1,6-glucan on the antioxidant and digestive enzyme activities of Pacific red snapper (*Lutjanus peru*) after exposure to lipopolysaccharides

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Abstract The effect of β-1,3/1,6-glucan, derived from yeast, on growth, antioxidant, and digestive enzyme performance of Pacific red snapper *Lutjanus peru* before and after exposure to lipopolysaccharides (LPS) was investigated. The β-1,3/1,6-glucan was added to the basal diet at two concentrations (0.1 and 0.2 %). The treatment lasted 6 weeks, with sampling at regular intervals (0, 2, 4, and 6 weeks). At the end of this period, the remaining fish from either control or β-glucan-fed fish were injected intraperitoneally with LPS (3 mg kg^{-1}) or with sterile physiological saline solution (SS) and then sampled at 0, 24, and 72 h. The results showed a significant increase ($P < 0.05$) in growth performance after 6 weeks of feeding with β-glucan. Superoxide dismutase (SOD) activity in liver was significantly higher in diets containing 0.1 % β-glucan in weeks 4 and 6, compared to the control group. β-Glucan supplementation at 0.1 and 0.2 % significantly increased aminopeptidase, trypsin, and chymotrypsin activity. At 72 h after injection of LPS, we observed a significant increase in catalase activity in liver from fish fed diets supplemented with 0.1 and 0.2 % β-glucan; SOD activity increased in fish fed with 0.1 % β-glucan in relation to those injected with SS. Feed supplemented with β-1,3/1,6-glucan increased growth, antioxidant activity, and digestive enzyme activity in *Pacific* red snapper.

Keywords β-1,3/1,6-Glucan · Antioxidant enzymes · Digestive enzymes · *Lutjanus peru* · Lipopolysaccharides

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Introduction

Pacific red snapper (*Lutjanus peru*), a native species in the eastern Pacific from Mexico to Peru (Allen 1985), has great demand and supports a large fishery in Mexico (FAO 2012). Despite its importance, assessment studies of the Pacific red snapper are scarce. Farming is an alternative to the threat of overfishing (FAO 2006). Increasing production of fish can also create stress, affecting the immune system and increasing the vulnerability to infectious diseases (Kumari and Sahoo 2006a, b), which leads to substantial economic losses from massive mortality.

Restriction or ban of antibiotics in fish and crustacean feed has prompted research toward alternative products for health management, such as prebiotics

(Kiron 2012). The benefits have been cited by many authors (Douglas and Sanders 2008; Reid 2008; Wang et al. 2008). Prebiotics are non-digestible (by the host) food ingredients that benefit the host by selectively stimulating the growth and/or the activity of select bacteria that improves the health of the host (Gibson and Roberfroid 1995).

β-1,3/1,6-Glucan, one the most studied prebiotics, is composed of a heterogeneous group of glucose polymers consisting of a backbone of β-1,3-linked β-D-glucopyranosyl units with β-1,6-linked side chains of varying distribution and length. Such patterns are abundant in microbial communities of prokaryotes and can be termed "pathogen-associated molecular patterns" (PAMP; Dalmo and Bøgwald 2008) that play a role as alarm molecules to activate the immune system (López et al. 2003; Aderem and Ulevitch 2000), increase antioxidant activity (Kumari and Sahoo 2006a), promote the growth of aquatic animals (Del Río et al. 2011), and confer protection against pathogenic organisms (Rodríguez et al. 2009; Dalmo and Bøgwald 2008; Ai et al. 2007).

Prebiotics offer an alternative method of manipulating and stimulating favorable indigenous microbial populations to enhance digestive functions of host by producing exogenous digestive enzymes and vitamins, which may help to maintain health and adequate nutrition in the cultivated organisms. Despite the promising benefits cited in the literature, obtaining consistent and reliable results is often difficult because there is an incomplete understanding of the gastrointestinal tract microbiota and their subsequent host interactions (Dimitroglou et al. 2011). Until now, reports related to the effect of β-glucan in fish have mainly focused on the immunological system but not on digestive enzyme activities. Effects on the physiology of digestion are not clear. β-Glucan is a non-sugar polysaccharide and a non-digestible material because β-glucanases are scarce or non-existent in fish. Despite this, glucans cannot be used as an energy source in fish, yet they play an important role in the regulation of the immune system and confer protection against pathogens. Knowledge of their effects on gut physiology is scarce or not available (Sinha et al. 2011).

In this study, we determined the influences of dietary β-1,3/1,6-glucan on growth, antioxidant, and digestive enzymes of *L. peru* after 6 weeks of supplementation, followed by exposure to LPS.

Materials and methods

General maintenance

We collected 153 juvenile Pacific red snapper from local fishermen. The fish were captured near Isla Cerralvo (∼24.25°N, ∼109.85°W), in the State of Baja California Sur, and transported to tanks at CIBNOR. They were acclimated for 8 weeks before starting the experiment. During acclimation, the fish were treated with a 10 % formaldehyde solution for 1 h to remove ectoparasites from the gills. The fish showed no pathological symptoms caused by infections or illnesses or disinfectant.

Fish were kept in nine 500-L tanks with an open circulation system with a flow rate up to 500 % of recycled and constantly aerated, filtered water each day, and exposed to natural photoperiod. Temperature (27 ± 1.5 °C), salinity (40 ± 0.83 g mL^{-1}), and dissolved oxygen (6.8 ± 0.75 mg O$_2$ L^{-1}) were measured every 2 days. The tanks were cleaned twice a day, including removal of fecal matter.

Diets and β-1,3/1,6-glucan

Three experimental diets were prepared from a commercial, pelleted feed (Skretting, Stavanger, Norway). Feed was pulverized and then reconstituted by adding 2 % alginic acid (#A7128, Sigma, St. Louis, MO) as a binding agent, and particles were mixed for 15 min, slowly and carefully adding the β-1,3/1,6-glucan (Fibosel®, Lallemand, Montreal, QC) to ensure effective incorporation and assure the desired final concentrations of 0.1 and 0.2 %. The control diet was not supplemented with β-glucan. Water was added (40 %), and the feed was pelleted (size 1 cm). Ingredients used for experimental diets are listed in Table 1. The proximate chemical composition of the main ingredients in the diets was analyzed according to the Official Methods of Analysis (AOAC 1990; Table 1).

Experimental design

Red snapper ($n = 153$; initial mean weight 340.38 ± 28.39 g; standard length 23.20 ± 1.06 cm) were randomly divided into three treatment groups (17 fish per group, each group in triplicate), based on the concentration of β-1,3/1,6-glucan added to the feed:

Table 1 Composition of diets used in treatments with different concentrations of β-1,3/1,6-glucan and chemical analysis used the following AOAC methods (1990)

Proximate composition	Control (0.0 % β-glucan)	β-Glucan 0.1 %	β-Glucan 0.2 %
Protein[a]	52.84 ± 0.1	52.79 ± 0.21	52.69 ± 0.17
Lipids[a]	23.25 ± 0.06	22.62 ± 0.27	22.78 ± 0.29
Crude fiber[a]	0.17 ± 0.04	0.16 ± 0.05	0.20 ± 0.07
β-1,3/1,6-Glucan from Fibosel[®b]	Not used	0.1	0.2

[a] All diets used a commercial food from SKRETTING (Vancouver, Canada) containing fish meal, poultry meal, corn gluten meal, fish oil, poultry fat, whole wheat, feather meal, lysine hydrochloride, polysorbate 60, a vitamin premix containing: vitamin A acetate, vitamin D, vitamin E, inositol, calcium pantothenate, riboflavin, nicotinic acid, thiamine mononitrate, pyridoxine hydrochloride, cobalamin, D-Biotin, folic acid, ascorbyl polyphosphate, menadione sodium, and bisulfite complex (vitamin K); DL methionine, a mineral premix containing manganese, sulfate, zinc methionine, calcium iodate, copper, sulfate, ferrous sulfate, sodium selenite, and ethoxyquin (antioxidant)

[b] Fibosel® manufactured by LALLEMAND

0.0 % (control), 0.1, and 0.2 %. The fish were fed twice each day to apparent satiation up to a limit of 2 % of biomass per day for 6 weeks. The feeding times and β-glucan doses were based on previous work (Berg et al. 2006; Bricknell and Dalmo 2005).

From the initial 153 fish, 99 were sampled during the 6 weeks of the trial. Of the remaining fish, 36 fish (12 fish per treatment) were injected intraperitoneally (3 mg kg^{-1}) with LPS from *E. coli* (#0127B8, Sigma) and 18 fish (6 fish per treatment group) were injected with a SS as a control.

Sampling

During the feeding period with β-1,3/1,6-glucan, samples were taken from 27 fish (9 fish per treatment) in the morning before feeding in weeks 0, 2, and 4. At the week 6, we collected 18 fish (6 fish per treatment) for further analysis. After 6 weeks, fish were exposed to LPS, and we sampled 12 fish per treatment (6 fish were injected with LPS and 6 fish injected with SS). Samples were taken at three intervals: just before the injection and after injection at 24 and 72 h.

Before blood collection, all fish were anesthetized with eugenol (#E-5504, Sigma) (1 mL eugenol in

30 L water). Blood samples were collected from the caudal vein with a 21-gauge needle and 3- and 5-mL syringes. The samples were centrifuged at 12,000*g* for 15 min at 4 °C to separate the plasma and then stored at −80 °C until analyzed. A portion of the liver was extracted to test for antioxidant enzymes, and a segment of the anterior intestine was tested for digestive activity. All samples were stored at −80 °C until analyzed.

Blood assays

Plasma protein content was measured by the method described by Bradford (1976). Total soluble protein concentration was read at 595 nm, according to the assay kit (#500-0006, Bio-Rad Laboratories, Hercules, CA). Bovine serum albumin (#A-4503, Sigma) was used to build a standard curve for determining protein concentration in each sample. Total hemoglobin levels in fresh blood were determined by spectrophotometric analysis at 540 nm, following the reaction with Drabkin's reagent (Drabkin and Austin 1935).

Growth performance

Body weight and length of each fish were measured, and growth was monitored in terms of percentage weight gain (%WG), specific growth rate (SGR), and condition factor (CF), which were calculated for each of the treatments: CF = (weight/fork length^3) × 100; SGR = [Ln (final weight − initial weight)/number of days] × 100; and %WG = (final weight − initial weight) × 100 (Silva-Carrillo et al. 2012).

SOD and CAT assays

SOD and CAT activities were assayed from supernatants derived from liver samples. Briefly, 0.1 g of tissue was homogenized in four volumes (v/w) of cold distilled water (4 °C) and centrifuged at 12,000*g* for 10 min at 4 °C. Supernatant was recovered and stored at −80 °C until analyzed.

The enzymes CAT and SOD were assayed in triplicate, using 10 and 20 μL of homogenates, respectively. CAT activity was assessed by measuring the decrease in absorbance at 240 nm caused by the disappearance of H_2O_2 during 1 min (Beauchamp and Fridovich 1971). SOD activity was assayed according to McCord and Fridovich (1969), where SOD

competes with cytochrome c for superoxide ions generated by hypoxanthine and xanthine oxidase reactions. SOD activity is determined by the level of reduction in inhibition of cytochrome c at 550 nm. Activities were reported as unit mg^{-1} protein. Protein concentrations in each sample were determined according to Bradford (1976).

Digestive enzyme activity

Each intestinal segment was homogenized in four volumes (v/w) of cold distilled water (4 °C) and centrifuged at 12,000g for 10 min at 4 °C. Supernatant was recovered and stored at −80 °C until analyzed to determine enzymatic activity, which was measured only during the feeding period before exposure to LPS.

Trypsin activity was measured at 25 °C, as described by Elanger et al. (1961), using N-α-ben-zoyl-DL-arginine 4-nitroanilide hydrochloride (BAP-NA) as substrate in 10 mM dimethyl sulfoxide (DMSO) and 50-mM Tris–HCl buffer with 10 mM CaCl$_2$ at pH 8.2. Chymotrypsin activity was measured at 25 °C, as described by Del Mar et al. (1979), using N-succinyl-ala-ala-pro-phe p-nitroanilide (SAAPNA) as substrate in 10 mM DMSO and 100 mM Tris–HCl buffer with 10 mM CaCl$_2$ at pH 7.8. Aminopeptidase was determined at 25 °C, as described by Maraux et al. (1973), using 50 mM sodium phosphate buffer at pH 7.2 and leucine p-nitroanilide as substrate in 0.1 mM in DMSO. For all these enzymes, the reactions were stopped by adding 30 % acetic acid. One unit of enzyme activity was defined as 1 µmol of p-nitroan-iline released per minute using coefficients of molar extinction of 8.2 mL^{-1} µmol^{-1} cm^{-1} at 410 nm. Alkaline phosphatase was assayed at 25 °C, as described by Bergmeyer (1974) by incubating the extract with 2 % (v/v) 4-nitrophenyl phosphate in acid citrate buffer at pH 5.5. After 30 min, 0.05 N NaOH was added and absorbance measured at 405 nm. One unit was defined as 1 µg nitrophenyl released per minute using a molar extinction coefficient of 18.5 at 405 nm. All assays were performed in triplicate. The specific activity of extracts was determined using the following equations: (1) Units mL^{-1} = (Δabs × reaction final volume (mL)/[MEC-time (min) − extract volume mL^{-1}]; (2) Units mg^{-1} of protein = Units mL^{-1} mg^{-1} of soluble protein. Δabs represents change in absorbance at a determined wavelength, and MEC represents the molar coefficient extinction for the product of the reaction (mL^{-1} µmol^{-1} cm^{-1}). Digestive enzyme activities were expressed as U mg protein^{-1}.

Statistical analysis

Experimental parameters (hematological index, antioxidant and digestive enzyme activity, and growth) were measured in triplicate. To determine data normality, we used the Kolmogorov–Smirnov test. Data from each treatment were subjected to one-way ANOVA with significance set at $P < 0.05$ using SPSS 13.0 statistical software (IBM SPSS, Armonk, NY). The results were presented as mean ± SD. When overall differences were significant, Tukey's multiple range tests were used to compare the means between individual treatments.

Results and discussion

Dietary effects of β-1,3/1,6-glucan

Blood assays

Hematological data for juvenile *Lutjanus peru* ($n = 27$) raised in the laboratory before the feeding trial are shown in Table 2. While hematological parameters were used to detect physiological changes (Atamanalp and Yanik 2003), in our study, hemoglobin and total plasma protein levels were not affected ($P > 0.05$) by either level of β-glucans (data not shown).

Blood parameter values of fish in the three treatments did not indicate any adverse effect on the health of *L. peru* and were similar to those reported for

Table 2 Hematologic values and antioxidant enzymes for juvenile red snapper *Lutjanus peru* raised in the before the feeding trial ($n = 27$)

Component	Mean ± SD
Hemoglobin (g dL^{-1})	8.10 ± 2.32
Plasma protein (mg mL^{-1})	41.27 ± 0.51
CAT (U mg protein^{-1})	1.70 ± 0.74
SOD (U mg protein^{-1})	11.42 ± 5.69

Values are given as mean ± SD for 27 individuals

SOD superoxide dismutase, *CAT* catalase

Table 3 Growth performance of juvenile red snapper *Lutjanus peru* fed diets supplemented with β-1,3/1,6-glucan at 0.0 % (control), 0.1, and 0.2 %

Experimental diets	Wi	Wf	SGR	WG (%)	FC
Control	350.08 ± 20.81a	405.68 ± 31.95b	0.34 ± 0.08b	15.83 ± 4.24b	2.89 ± 0.55a
β-Glucan (0.1 %)	333.67 ± 51.41a	520.26 ± 58.21a	1.06 ± 0.11a	56.79 ± 7.64a	2.99 ± 0.22a
β-Glucan (0.2 %)	337.40 ± 12.96a	493.56 ± 47.27ab	0.89 ± 0.15a	46.09 ± 9.61a	2.91 ± 0.05a

Values are expressed as mean ± SD of triplicate observations ($n = 9$)

Means in the same row with different scripts were significantly different ($P < 0.05$)

Wi initial weight, *Wf* final weight, *SGR* specific growth rate, *WG (%)* weight percentage gain, *CF* condition factor

clinically healthy fish in tropical climates and from spotted rose snapper *Lutjanus guttatus* fed with 0.1 % β-glucan supplement (Del Río-Zaragoza et al. 2011).

Growth performance

The fish fed β-1,3/1,6-glucan at either concentration had the highest growth ($P < 0.05$) in SGR and %WG in relation to control group (Table 3). In a comprehensive review on immunostimulants (Dalmo and Bowald 2008), the effects of β-glucan on fish growth are described. β-Glucans obtained from yeast cell walls increased the growth rate of tested fish species under specified temperature conditions (Sealey et al. 2008; Misra et al. 2006); however, in other studies under different conditions, no significant growth increase was found at several levels of β-glucan administration (Welker et al. 2007; Whittington et al. 2005).

The best growth was obtained in fish fed a supplement of 0.1 % β-glucan, followed by those fed a supplement of 0.2 % β-glucan. Authors report improved growth performance in fish, such as silver seabream *Chrysophrys auratus* (Cook et al. 2003) and in *L. guttatus* (Del Río et al. 2011) fed a supplement of 0.1 % glucan. Enhanced growth of *L. peru* in our experiment could be attributed to extracellular enzymes from the gut microflora (Dimitriglou et al. 2011). In contrast, there was no enhancement of growth in other fish species fed supplements of β-glucan, such as common dentex *Dentex dentex* (Efthimiou 1996).

Increase in growth is dependent on the amount of β-glucan in the diet, duration of feeding, environmental temperature, and the species under study (Dalmo and Bøgwald 2008). Our results showed a significant increase in growth in fish treated with β-1,3/1,6-glucan. This has potential to reduce feeding costs in marine fish pond farming.
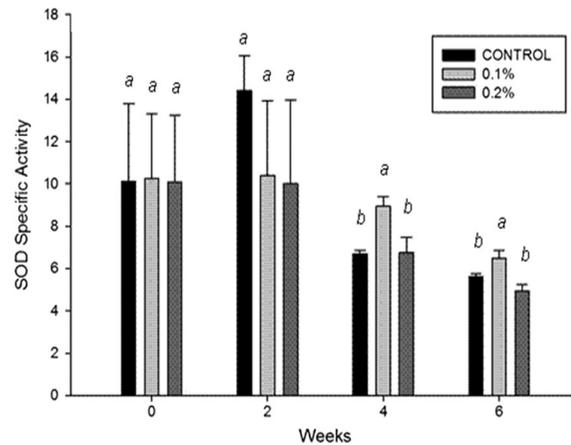

Fig. 1 Effect of β-1,3/1,6-glucan on activity of superoxide dismutase. Data are mean ± SD. *Different superscripts* indicate statistical significance ($P < 0.05$; $n = 9$)

Antioxidant enzyme activities

During the trial, catalase activity showed no significant differences (data not shown). SOD activity was significantly higher in diets containing 0.1 % β-glucan (Fig. 1), in weeks 4 and 6, compared to the control group. Our results are similar to other experiments, where an increase in SOD in fish fed diets containing different types and concentrations of β-glucans (Kumari and Sahoo 2006a) or methods of administration (Selvaraj et al. 2005) for species, such as rohu *Labeo rohita* (Sahoo and Mukherjee 2001; Misra et al. 2006), Asian catfish *Clarias batrachus* (Kumari and Sahoo 2006b), and common carp *Cyprinus carpio* (Selvaraj et al. 2005). Increased SOD activity could be explained by the role of β-glucan to increase the number of circulating neutrophils (Del Río et al. 2011) and activate phagocytic cells associated with responses to reactive oxygen species (ROS; Swain et al. 2008) and increase antioxidant enzymes to counteract harmful effects of ROS (Kumari and Sahoo 2006a; Muñoz et al. 2000).

Digestive enzyme activity

We found a significant increase in trypsin activity when fish received a supplement of 0.1 % β-glucan at week 2 of feeding (Fig. 2a); at week 4, chymotrypsin activity increased in fish that received 0.2 % β-glucan (Fig. 2b). Aminopeptidase activity increased in fish that received 0.1 and 0.2 % β-glucan at week 4 (Fig. 2c). Alkaline phosphatase activity did not differ in any treatment (data not shown).

Most studies dealing with β-1,3/1,6-glucan in fish focus on the immunostimulant effect (Dalmo and Bøgwald 2008). So far, few reports explore the digestive process, including absorption or digestibility of β-glucan in the fish intestine (Krogdahl et al. 2005; Dimitroglou et al. 2011; Pedrotti et al. 2013).

Our results agreed with Xu et al. (2009), where carp that were given xylo-oligosaccharides (1.5 mg kg^{-1}) respond with an increase in digestive activity of protease. Thus, better enzyme activity is associated with the beneficial change in the carp's gut flora. Tovar et al. (2004) reports improved activity of trypsin in European sea bass *Dicentrarchus labrax* larvae by adding live yeast *Debaryomyces hansenii* to the diet. In our work, the beneficial influence of β-glucan on digestive enzyme activities could be possibly due to an alteration of the intestinal microbiota. However, a detailed study of intestinal microbiota is needed.

From another point of view, Anguiano et al. (2012) report that improvements in nutrient digestibility by prebiotic supplementation appear to be mostly related to changes in the gastrointestinal tract structure but did not find enhancement of digestive enzyme activity.

Other studies were non-related to fish, as that of Gaggìa et al. (2010) who found that pigs fed β-glucan have increased digestive enzyme activities such as amylase and protease. Iji et al. (2001) reported that broiler chicks responded to diets supplemented with commercial non-sugar polysaccharides, observing that the activity of aminopeptidase N in the ileum was also stimulated by a highly viscous non-starch polysaccharide. This report coincides with our results, concerning the increase in aminopeptidase activity in the intestine of fish fed β-glucan supplementation at 4 weeks of feeding.

Dimitroglou et al. (2011) suggest that some bacterial populations contribute to modify the host's digestive enzyme activity through its ability to produce and liberate exogenous digestive enzymes. Other studies (Ringφ and Olsen 1999; Li and Gatlin 2005) find that polysaccharides used as prebiotics stimulate growth of beneficial microbiota in fish. A study by Bairagi et al. (2002) of microbiota in nine species of teleosts found that the microbiota is a source of exogenous digestive enzymes in addition to the endogenous enzymes produced in the gastrointestinal tract and identify these bacteria for their ability to produce lipase, protease, cellulose, and trypsin. They did not identify these bacteria with molecular methods.

Exposure to lipopolysaccharides (LPS)

Blood assays

Hemoglobin in fish injected with LPS showed no significant changes (data not shown); however, at 72 h, the concentration of plasma proteins significantly decreased ($P < 0.05$) in fish fed with control diet and increased ($P < 0.05$) in fish fed with β-glucan at 0.1 %, compared to those injected with SS (Fig. 3).

LPS affects hematological parameters and induces acute stress (Swain et al. 2008), causing hematopoietic proliferation (MacKenzie et al. 2008). Our data on total serum proteins is a reflection of the innate immunity (Wiegertjes et al. 1996). The decrease in plasma proteins in fish fed control diet may be because these physiological and biochemical responses vary depending on species and endotoxin doses administered (Huttenhuis et al. 2006; Selvaraj et al. 2005), so further studies are needed to clarify the effects of LPS and glucans on hematological parameters in Pacific red snapper.

Antioxidant enzymes activities

At 24 h, fish fed the control diet and injected with LPS had significantly lower CAT activity compared to fish injected with SS (Fig. 4a). This does not agree with some reports, where antioxidant enzyme activities increase in fish subjected to an acute stressor or exposed to toxins, such as LPS (Swain et al. 2008; Nayak et al. 2008; Guttvika et al. 2002). Nevertheless, at 72 h, CAT activity increased in fish receiving β-1,3/1,6-glucan at 0.1 and 0.2 % (Fig. 4b), compared to those injected with SS. This confirms that these specific polysaccharides increase antioxidant activity in fish (Dalmo and Bøgwald 2008).

At 72 h, our results showed a significant decrease in SOD in fish fed control diet and injected with LPS

Fig. 2 Effect of β-1,3/1,6-glucan on specific intestinal activity of trypsin (**a**), chymotrypsin (**b**), and aminopeptidase (**c**). Data represent mean ± SD. *Different superscripts* indicate statistical significance ($P < 0.05$; $n = 9$)
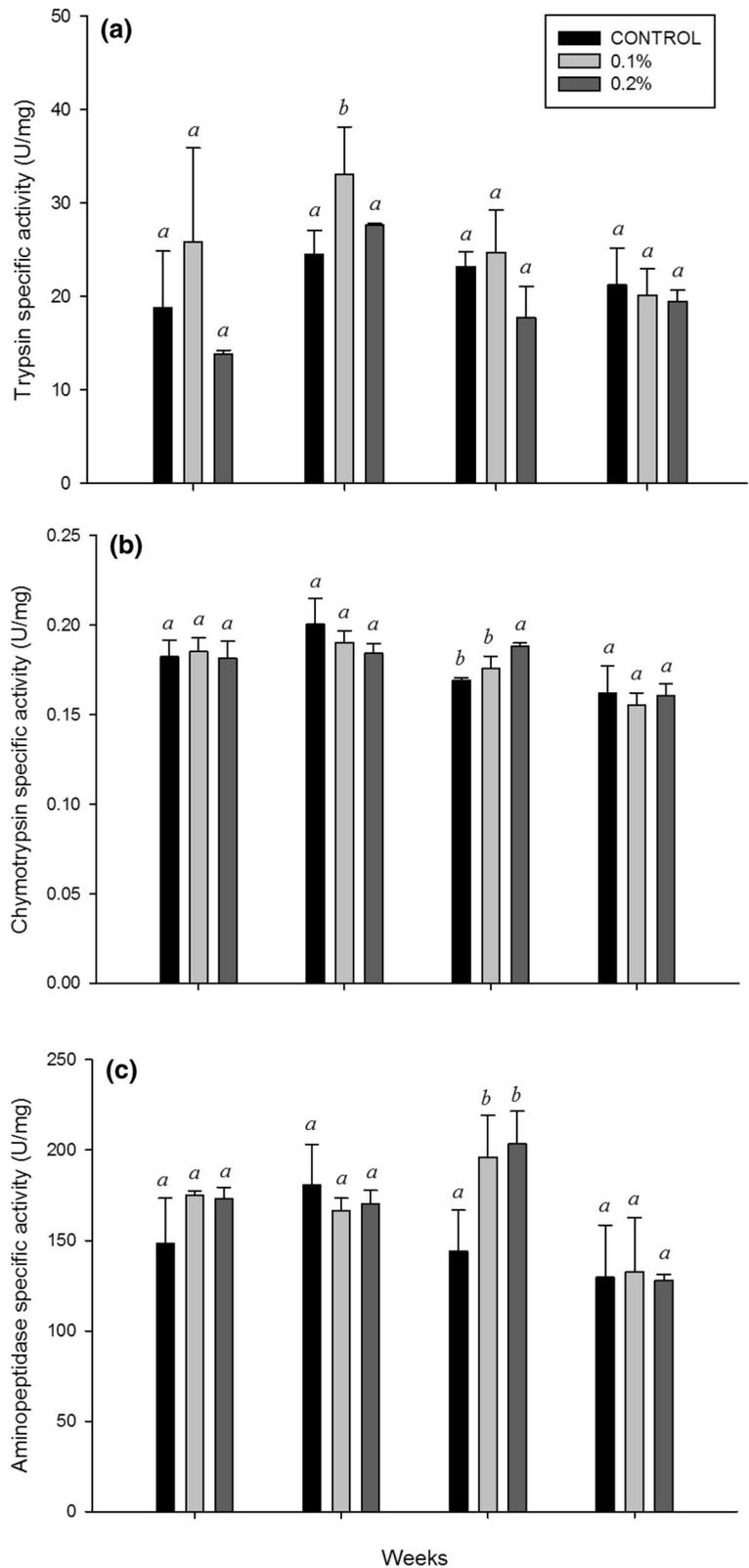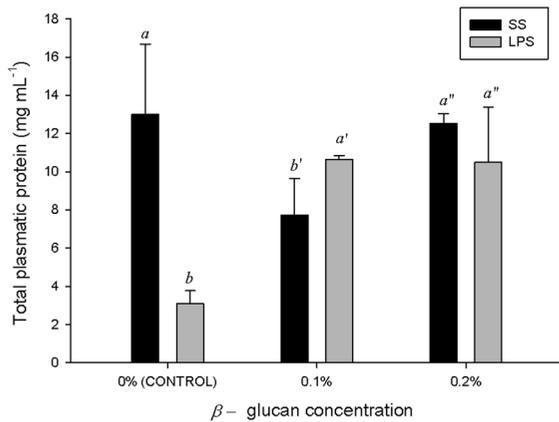
Fig. 3 Effect of β-1,3/1,6-glucan on the total plasma protein at 72 h after exposure to lipopolysaccharides (LPS). Data represent the mean ± SD. *Different superscripts* indicate statistical significance ($P < 0.05$) between sterile physiological saline solution (SS) and LPS treatments ($n = 6$)
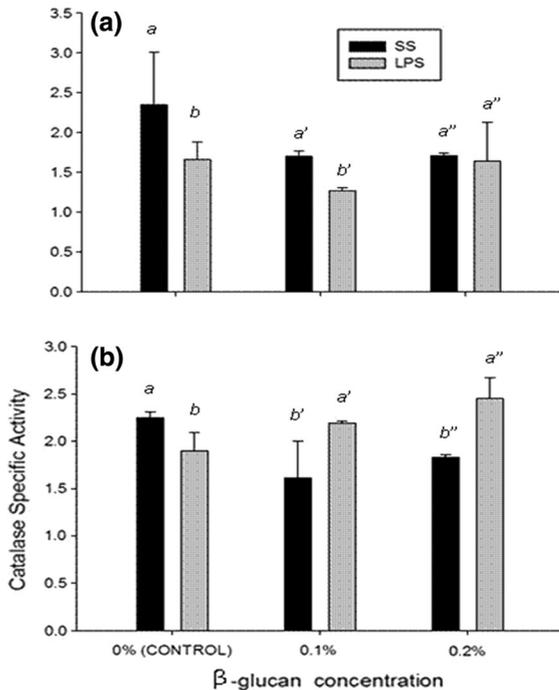

Fig. 4 Effect of β-1,3/1,6-glucan on catalase activity at 24 h (**a**) and 72 h (**b**) after exposure to lipopolysaccharides (LPS). Data represent the mean ± SD. *Different superscripts* indicate statistical significance ($P < 0.05$) between sterile physiological saline solution (SS) and LPS treatments ($n = 6$)

compared to those injected with SS (Fig. 5). It has been observed that the activity and expression of SOD are both tissue and dose-dependent when an organism is challenged with LPS. For example, Mn-SOD
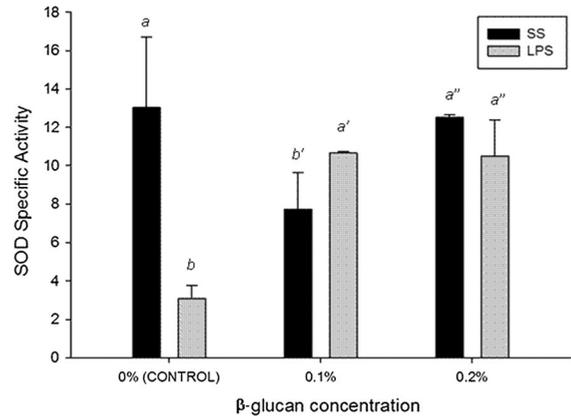

Fig. 5 Effect of β-glucan 1,3/1,6 on superoxide dismutase at 72 h after exposure to lipopolysaccharides (LPS). Data represent the mean ± SD. *Different superscripts* indicate statistical significance ($P < 0.05$) between sterile physiological saline solution (SS) and LPS treatments ($n = 6$)

expression in the liver and kidney was positively modulated by injection of LPS, but the level of transcription was inversely related to the strength of bacterial challenged in *Hemibarbus mylodon* (Cho et al. 2009).

Some studies state that LPS is a major virulence factor and responsible for lethal effects and clinical manifestations of diseases in humans and animals; however, in lower vertebrates, such as fish, they are often resistant to endotoxic shock (Brandtzaeg et al. 2001). This may explain our results in *L. peru*, although the immunostimulating effects of an endotoxin by triggering immune system-related cells, such as T and B lymphocytes and macrophages, have been established in several teleosts (Swain et al. 2008).

Also at 72 h, SOD activity increased in fish injected with LPS, compared to fish injected with SS and fed diets supplemented with β-1,3/1,6-glucans at 0.1 % (Fig. 5), which was not surprising because the enzyme is required to remove reactive free radicals that may be harmful to the fish (Harikrishnam et al. 2010). Additionally, increased SOD activity may result from stimulation of macrophages by β-1,3/1,6-glucans, which possess certain conserved chemical conformations that affect the immune system (Uchiyama 1982) and antioxidant enzymes (Dalmo and Bøgwald 2008; Selvaraj et al. 2006). SOD activity did not vary in fish treated with or without LPS and given 0.2 % β-glucan.

Increased production of superoxide anions occurs in many fishes exposed to LPS (Gutsmann et al. 2001) as was observed in our work and in different species

such as Atlantic salmon *Salmo salar,* seabream *Sparus aurata,* rainbow trout *Oncorhynchus mykiss,* european sea bass *Dicentrarchus labrax,* carp *Cyprinus carpio, and* goldfish *Carassius auratus* (Doñate et al. 2007; Goetz et al. 2004; Sarmento et al. 2004; Saeij et al. 2003; Zou et al. 2002; Engelsma et al. 2001).

Conclusion

This study clearly indicated that diets supplemented with β-1,3/1,6-glucan at a suitable concentration (0.1 and 0.2 %) improve growth, efficacy of SOD and CAT antioxidant enzymes before and after exposure to LPS, and stimulated digestive enzyme activities, including trypsin, aminopeptidase, and chymotrypsin in *L. peru*. The beneficial influence of β-1,3/1,6-glucan on digestive activity was likely a result of an alteration of the intestinal microbiota. These results can be used to aid in formulating supplements for red snapper that are cost-effective and provide a basis for understanding the effects of prebiotics on the production of digestive enzymes by microbiota.

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