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Effect of dietary mannan oligosaccharide (MOS) on growth, digestive enzymes activity, innate immunity and disease resistance against virulent *Aeromonas hydrophila* in peninsular carp *Barbodes carnaticus* (Jerdon, 1849)

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ABSTRACT

The effect of dietary mannan oligosaccharide (MOS) on growth, digestive enzymes activity, innate immunity and disease resistance in the Indian minor carp *Barbodes carnaticus* (Jerdon, 1849) was evaluated over a period of 90 days in soil based cement tanks. *B. carnaticus* recorded higher weight gain after feeding MOS incorporated diets, with no difference between 0.5 and 1% levels. The feed conversion ratio (FCR) and protein efficiency ratio (PER) were higher with MOS incorporated diets, with the lowest FCR and highest PER recorded by 0.5% MOS diet. Higher activities of hepatopancreatic and intestinal protease, lipase and amylase were recorded in *B. carnaticus* fed 0.5% MOS diet. The activity showed a decrease at 1% level of MOS incorporation. Carcass proximate composition analysis of *B. carnaticus* revealed no difference in the moisture, fat and ash contents. However, the crude protein content was higher with MOS treated fish. The group fed with 0.5% MOS recorded significantly higher respiratory burst, alternate haemolytic complement activity (ACH50), lysozyme activity, albumin/globulin (A:G) ratio, myeloperoxidase activity (MPO) and nitric oxide (NO) levels, followed by 1% MOS and Control diets, respectively. The higher relative percentage of survival (RPS) against virulent *Aeromonas hydrophila* challenge was observed in MOS fed group. The study revealed that MOS supplementation improves the digestive enzyme activity and has got beneficial effect on the growth of *B. carnaticus*.

Keywords: *Aeromonas hydrophila* challenge, *Barbodes carnaticus*, Digestive enzyme, Growth, Innate immunity, Mannan oligosaccharide

Introduction

Barbodes carnaticus (Jerdon, 1849) is endemic to the Western Ghats of India. It is a much preferred food fish and is caught from wherever they occur. The higher growth rate of *B. carnaticus* in the first year of its life span along with other favourable characteristics makes this species an excellent candidate for freshwater aquaculture (Manojkumar and Kurup, 2010). Although it is an excellent candidate for carp species diversification in Indian aquaculture, its relatively slower growth than Indian major carps (IMCs) is a limiting factor. In order to enhance its growth under culture conditions, a good probiotic/prebiotic which can exploit species potential is the need of the hour.

With the European moratorium on the use of antibiotics as growth promoters in animal and fish feeds, research on alternate feed additives has been major objective throughout the world towards inching up the quality and productivity in aquaculture. In this direction, prebiotics are emerging as ideal replacement for antibiotics as it addresses the issue of environmental and consumer concerns. The concept of prebiotics is a recent entry in the functional food science, which is expected to show its potentiality beyond the nutritional requirement by selective stimulation of gut microflora. The beneficial effects of prebiotics are not only limited to the gastrointestinal ecology, but also found in several physiological functions such as immune modulation, blood cholesterol regulation and bone mineralisation (Samanta *et al.,* 2013). Amongst the prebiotics, mannan oligosaccharides (MOS) are thought to be important as it is very well evaluated for growth and immune-stimulatory properties in different host species (Torrecillas *et al.,* 2014; Spring *et al.,* 2015). MOS has been demonstrated to enhance the growth of several fish genera including rainbow trout (Staykov *et al.,* 2007; Denji *et al.,* 2015), European seabass (Torrecillas *et al.,* 2007), rohu

(Andrews *et al*., 2009), freshwater crayfish (Mazlum *et al.,* 2011), yellow catfish (Wu *et al.,* 2014), Asian seabass (Ali *et al.,* 2017), stripped catfish (Akter *et al.,* 2016) and snakehead (Hien *et al.,* 2016). Further, MOS is found to be having immune-modulatory effect by stimulating innate immunity/non-specific immune responses necessary for disease resistance in fish (Andrews *et al.,* 2009; Peterson *et al.,* 2010; Sang *et al.,* 2011; Torrecillas *et al.,* 2011; Welker *et al.,* 2012; Liu *et al.,* 2013). There exists substantial difference and variations between fish species in terms of MOS dose/concentration, routes and magnitude of immune-modulatory effects (Torrecellas *et al.,* 2015). This study was conducted to evaluate the effect of MOS supplementation in the diet of Carnatic carp, *B. carnaticus* on growth, body composition, digestive enzyme activity, innate immune response and resistance against virulent *Aeromnas hydrophila* challenge.

Materials and methods

Experimental diets

A basal diet was formulated to contain 30% crude protein using locally available ingredients (Table 1). Experimental diets were prepared by adding 0.5 and 1% mannan oligosaccharide (MOS) obtained from *Saccharomyces cerevisiae* (Bio-MOS, AltechInc USA) to the basal (Control) diet. Ground nut oilcake and finger millet were dried, powdered and sieved through a fine meshed screen (0.5 mm). Required quantities of ingredients except vitamin and mineral mixture were mixed with hot water to make a dough. After cooling, vitamin and mineral mixture were added and mixed. The dough was pressed through a hand pelletiser to get uniform sized pellets (2 mm). The pellets were sun dried and packed in polythene bags till further use. Proximate composition of feed was determined following the standard methods (AOAC, 1995).

Table 1. Ingredient proportion (%) and proximate composition (Mean±SD) of experimental diets

Diets	Control	0.5% MOS	1% MOS
Ingredients (%)			
Fishmeal	5	5	5
Soybean cake	21	21	21
Groundnut cake	30	30	30
Rice bran	33	32.5	32
Finger millet	10	10	10
Vitamin and mineral mixture*	1	1	1
MOS	0	0.5	1.0
Proximate composition (%)			
Moisture	4.21±0.03	6.25±0.11	5.1±0.11
Crude protein	30.07±0.43	29.45±0.04	29.57±0.57
Fat	6.91±0.02	6.12±0.01	6.82±0.29
Ash	9.05±0.04	9.89±0.38	8.98±0.42
Crude fiber	9.39±0.88	9.32±0.01	9.5±0.008
NFE	40.37±1.51	38.77±0.69	40.03±0.89

*Vitamin and mineral mixture (Agrimin Forte, Virbac Animal Health, Mumbai). Per kg composition - Vitamin A-7,00,000 IU; Vitamin D_3-70,000 IU; Vitamin E-250 mg; Nicotinamide-1000 mg; Cobalt-15 mg; Copper-1200 mg; Iodine-325 mg; Iron-1500 mg; Magnesium-6000 mg; Potassium 100 mg; Sodium 5.9 mg; Manganese-1500 mg; Sulphur-0.72%; Zinc-9600 mg; Calcium-25.5%; Phosphorus12.75%.

Growth trial

Advanced fingerlings (average wt. 21.54 g) of *B. carnaticus* produced at the Bangalore Regional Research Centre of the ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA), were randomly distributed in nine cement tanks (4×4×1.2 m) at a stocking density of 16 fish per tank (10,000 ha^{-1}). Cement tanks were prepared before water was filled by providing a bottom soil layer of 3 inch thickness followed by liming of the tanks with lime at 200 kg ha^{-1}. Tanks were randomly grouped into three sets for feeding either of the diets - Control (0), 0.5 and 1% MOS. Feeding of fish at 10.00 hrs at 5% of the body weight and collection of the left over feed next morning before feeding were performed on daily basis up to 90 days. Water quality parameters of the tanks were measured at fortnightly basis for pH, temperature, dissolved oxygen, total alkalinity, ammonia, nitrate and phosphate (APHA, 1998). On termination of the experiment, fish were weighed individually and survival recorded. Feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR), condition factor and production were also calculated. The proximate composition of experimental fish carcass was determined following standard methods (AOAC, 1995).

The ethical guidelines of the Institute Animal Ethical Committee (IAEC), ICAR-CIFA, Bhubaneswar were followed throughout the investigation.

Preparation of enzyme extract

Gut and hepatopancreas of six fish from each treatment were dissected out and macerated separately with four times volume of ice cold distilled water and centrifuged at 16,000 rpm for 20 min at 4°C. The extract was decanted and the pellet re-suspended in equal volume of cold distilled water and centrifuged as before. The washing procedure was repeated and washings were combined with the first extract. The crude enzyme extract thus obtained was divided into 1 ml aliquots and stored at -20ºC. All extraction procedures were carried out at 4ºC. Protein in the crude enzyme extracts was estimated according to Lowry *et al.* (1951) using bovine serum albumin (fraction V) as standard.

Enzyme assays

All assays were carried out separately for both gut and hepatopancreatic enzymes. Amylase activity was

assayed against soluble starch. The reaction mixture consisted of 25 µl of crude enzyme extract and 250 µl of 1% starch dissolved in 0.1MTris-HCl, pH 7.0 buffer, pre-incubated at 37ºC. After 1 h, the reducing sugars formed were quantified by the Nelson-Somogyi method (Somogyi, 1952; Nelson, 1944) using appropriate blanks. Specific activity of amylase was expressed as µM of glucose liberated h^{-1} mg tissue $protein^{-1}$.

Total proteolytic activity was determined by the casein digestion method (Kunitz, 1947) using 1% solution of casein (SRL Laboratories, Mumbai) as the substrate. The reaction mixture consisted of 0.1 ml of crude enzyme extract added to pre-warmed substrate buffer at 37ºC and incubated for 30 min. At the same temperature, the reaction was terminated by addition of 3 ml of ice cold 5% tricholoro acetic acid and allowed to stand at 4ºC for 1 h. The absorbance of the supernatant obtained by centrifugation of the reaction mixture for 20 min at 15000 rpm was determined at 280 nm. A standard curve was drawn with tyrosine and enzyme activity expressed as µM of tyrosine liberated h^{-1} mg tissue $protein^{-1}$.

Trypsin activity was assayed according to the method of Erlanger *et al.* (1961) against N-α-benzoyl-DL arginine-p-nitroanilide hydrochloride (BAPNA) as the substrate. The activity of trypsin was calculated from the increase in absorbance at 410 nm for 10 min at 25ºC using p-nitroaniline as the standard using a spectrophotometer. Activities of trypsin were expressed as µM of p-nitroaniline liberated h^{-1} mg of tissue $protein^{-1}$.

Lipase activity was determined using p-nitrophenyl acetate (PNPA) as the substrate according to Licia *et al.* (2006). Buffered substrate (2.9 ml) was added to 0.1 ml of crude enzyme extract and incubated at room temperature for 10 min. Absorbance was measured at 410 nm using spectrophotometer. Activities of lipase were expressed as µM of p-nitrophenol liberated h^{-1} mg of tissue $protein^{-1}$.

Innate immune response

To measure the status of innate immunity, the following tests *viz,* nitro blue tetrazolium (NBT) reduction assay; myeloperoxidase enzyme activity assay (MPO); serum albumin/globulin ratio (A:G) assay; nitric oxide (NO) assay; alternate haemolytic complement activity (ACH50) and lysozyme activity, were performed. On termination of the experiment, 0.2 ml of blood with heparin for NBT assay and 0.5 ml without heparin for serum separation was collected from at least 15 fish per treatment. NBT assay, MPO assay, NO assay, ACH50 and lysozyme activity were evaluated as per the standard protocols with partial modifications.

NBT assay

Respiratory burst activity (RBA) in the blood was evaluated by NBT assay as per the methods described by Sahoo *et al.* (2005). For this, about 0.1 ml of heparinised blood from experimental samples was mixed with 0.1 ml of 0.2% freshly prepared filtered NBT in normal saline and incubated at 37°C for 30 min. About 50 µl of resultant suspension was mixed with 1ml of N, N-dimethyl formamide and centrifuged at 3000 *g* for 5 min. The optical density (OD) of the supernatant was measured at 540 nm in a spectrophotometer (Bio-Rad Smart SpecTM 3000).

MPO activity

The level of MPO in serum was estimated as per the method described by Quade and Rath (1997) and tested by Lee *et al.* (2018). For estimation of MPO, 10 µl of serum was diluted with 90 µl of Hank's balanced salt solution (HBSS) without Ca^{2+} and Mg^{2+} in 96 well micro-titer plates. To this, about 35 µl of 3', 3'5',5'- tetra-methyl benzidine hydrochloride (TMB) and 5 mM H_2O_2 was added and change in colour reaction was stopped by adding 35 µl of 4 M sulphuric acid. The OD was recorded at 450 nm in microplate reader.

Nitric oxide production assay

Indirect methods were used to obtain NO level in the serum by estimating nitrite/nitrate using Griess reaction as per the methods described by Das *et al.* (2018). To estimate nitrite level in the serum, about 100 µl of serum was diluted with 400 µl of carbonate buffer. The resulting solution was mixed with 100 µl of Griess reagent and incubated at room temperature for 30 min. The reaction was stopped by adding 0.35 M of sodium hydroxide. The optical density of the resultant colour reaction was obtained at 545 nm in a microplate reader in 96 well plate. The concentration was determined from the standard curve generated for sodium nitrite.

Albumin:globulin (A:G) ratio

A:G ratio in serum samples was estimated by determining albumin concentration using Bromocresol green method. Bromocresol green has high affinity to albumin and turns blue once it binds with albumin. The concentration of albumin was estimated by measuring the absorbance at 540 nm in spectrophotometer. A standard curve was generated with bovine serum albumin (BSA) by estimating protein concentration by Lowry method. A:G ratio was calculated by the method of Andrews *et al.* (2009).

ACH50 titre

ACH50 in the serum of experimental fish was calculated as per the method described and modified by Kumari and Sahoo (2006). The 50% haemolysis was expressed as ACH50 (units ml^{-1}) from the graphs following Matsuyama *et al.* (1988) and Yano (1992).

Lysozyme activity

Lysozyme activity in the serum of experimental fish was estimated as per the method described by Kumari and Sahoo (2005). Efficiency of enzyme activity was calculated by the ability to kill *Micrococcus lysodeikticus* and was measured by turbidometry assay at OD 450 nm. Results were expressed as µg ml^{-1} enzyme activity with hen egg white lysozyme as standard.

Challenge study

At the end of the experiment, 10 fish per treatment were experimentally challenged with virulent *A. hydrophila* (obtained from Fish Health Management Laboratory, ICAR-CIFA, Bhubaneswar, India) at the dose standardised for *B. carnaticus* (10^6 CFU ml^{-1}) through intra-peritoneal route. The survival rate after 10 days post-challenge was analysed by Kaplan-Meier method in SPSS software.

Statistical analysis

Comparison among treatments for various parameters was done by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (Duncan, 1955; Snedecor and Cochran, 1968).

Results

Water quality parameters

Water quality parameters analysed during the experimental period were within the favourable limits; Temperature (ºC): 27.41±1.00 (25-29); pH: 8.23±0.37 (7.78-8.86); Alkalinity (mg l^{-1}): 226.35±22.20 (195.52-263.2); DO (mg l^{-1}): 6.82±1.58 (3.2-11.2); Hardness (mg l^{-1}): 213.06±35.59 (148-248); Phosphate (mg l^{-1}): 0.31±0.33 (0.07-0.68); Ammonia (mg l^{-1}): 0.09±0.18 (0-0.75); Nitrate (mg l^{-1}): 0.55±0.12 (0.35-0.71).

Growth and enzyme activity

B. carnaticus recorded higher ($p<0.05$) weight gain after feeding MOS incorporated diets, with no difference ($p>0.05$) between 0.5 and 1.0% levels (Table 2). The feed conversion and protein efficiency ratio were higher with MOS incorporated diets, with the lowest FCR and highest PER recorded by 0.5% MOS diet. The 'Condition factor', which is an index of the well-being of the fish under culture was higher for fish fed MOS. Fish in all the tanks survived as recorded at the end of the study period. Higher activities of hepato-pancreatic ($p<0.05$) and intestinal ($p>0.05$) protease, lipase and amylase were recorded in *B. carnaticus* fed 0.5% MOS diet (Fig. 1 and 2). The activity showed a decrease at 1% level of MOS incorporation. Carcass proximate composition analysis revealed no difference in the moisture, fat and ash contents (Table 3). However, the crude protein content was higher with MOS-treated fish.

Innate immune functions

The results of innate immune parameters are presented in Fig. 3. The experimental group fed with 0.5% MOS diet recorded significantly (<0.05) higher respiratory burst and myeloperoxidase activities followed by 1% MOS and control groups. MOS at 0.5% level exhibited significantly (<0.05) higher globulin fraction as compared to other two groups. Significantly ($p<0.05$) higher NO levels were exhibited by 0.5% MOS-fed group. Similarly, significantly ($p<0.05$) higher lysozyme activity was also recorded in the 0.5% MOS fed group followed by 1% and

Table 2. Growth parameters (mean±S.D.) of *B.carnaticus* fed experimental diets for 90 days

Treatment	Initial weight (g)	Final weight (g)	Weight gain (g)	Survival (%)	FCR	PER	SGR (%)	Condition factor	Production (g $tank^{-1}$ 90 $days^{-1}$)
Control	21.90±0.26	49.37±3.22^a	27.48±3.17^a	100	3.32±0.27^c	1.05±0.11^a	0.90±0.07^a	1.21	789.97±51.45^a
0.50% MOS	21.46±0.18	62.29±4.44^b	40.83±4.55^b	100	2.29±0.13^a	1.45±0.06^b	1.18±0.10^b	1.46	996.58±71.11^b
1% MOS	21.26±0.10	58.53±3.07^b	37.27±2.97^b	100	2.76±0.25^b	1.23±0.12^a	1.12±0.06^b	1.46	936.48±49.06^b

Figures in the same row having same superscripts do not differ significantly ($p>0.05$).

Table 3. Carcass proximate composition (mean±S.D.) of *B. carnaticus* fed MOS incorporated diets

Treatment	Moisture	Crude protein	Fat	Ash
Control	66.41±0.50^a	15.73±0.52^a	12.02±0.90^a	3.35±0.19^a
0.5% MOS	64.49±1.30^a	19.08±0.79^b	12.14±1.03^a	2.90±0.21^a
1% MOS	65.24±0.73^a	17.93±0.56^b	12.46±0.70^a	3.10±0.25^a

Figures in the same row having same superscripts do not differ significantly ($p>0.05$).

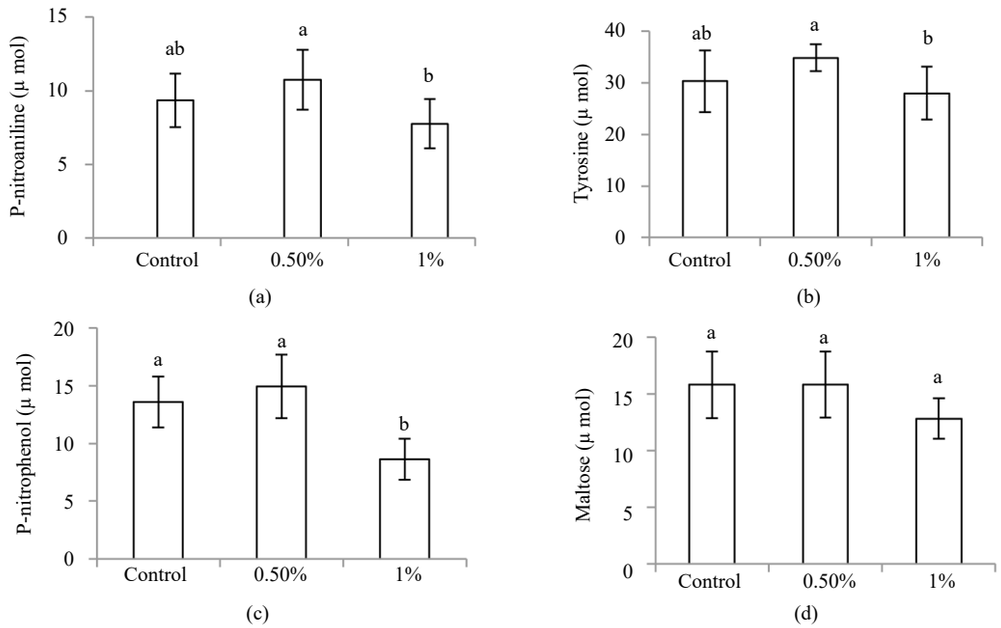

Fig. 1. Digestive enzyme activities (µm h^{-1} mg $protein^{-1}$ at 25°C) in the intestine of *B. carnaticus* fed experimental diets (a) Tyrpsin, (b) Total protease, (c) Lipase and (d) Amylase

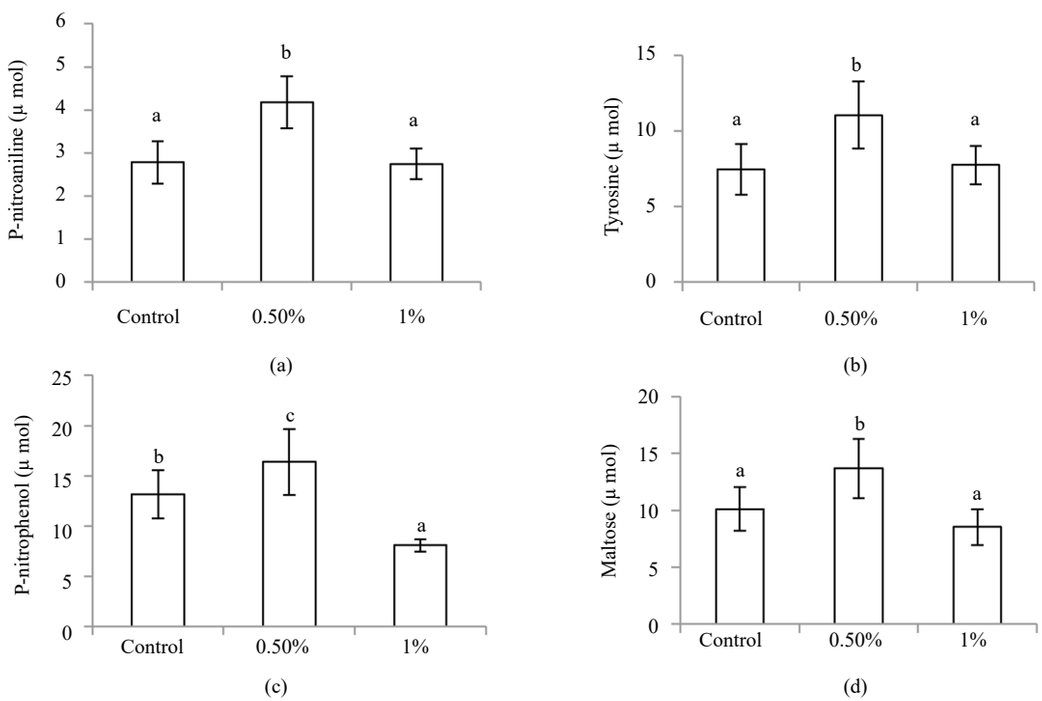

Fig. 2. Digestive enzyme activities (µM h^{-1} mg $protein^{-1}$ at 25°C) in the hepatopancreas of *B. carnaticus* fed experimental diets (a) Tyrpsin, (b) Total protease, (c) Lipase and (d) Amylase

control. Complement activity in serum of different groups was estimated and 0.5% MOS fed group was found to be having significantly higher ($p<0.05$) complement activity than other two groups.

Challenge study

In the challenge study, 0.5% MOS fed group showed significantly higher relative percentage survival (RPS) with 80% survival rate against virulent *A. hydrophila* than

1% MOS fed fish (70%) and 100% mortality was observed in the control group (Fig. 4).

Discussion

A need for diversification of farmed fish species has been emphasised (NACA/FAO, 2000). The minor carps can be considered as alternatives to the major carp species for diversification in freshwater aquaculture. It may be beneficial to enhance the growth of these minor carps by manipulation of nutrients/feed additives, considering their slower growth. MOS is an established prebiotic with growth enhancing, immune-potentiating and potentiating disease resistance properties in various fish species. In the present study, *B. carnaticus* recorded higher ($p<0.05$)

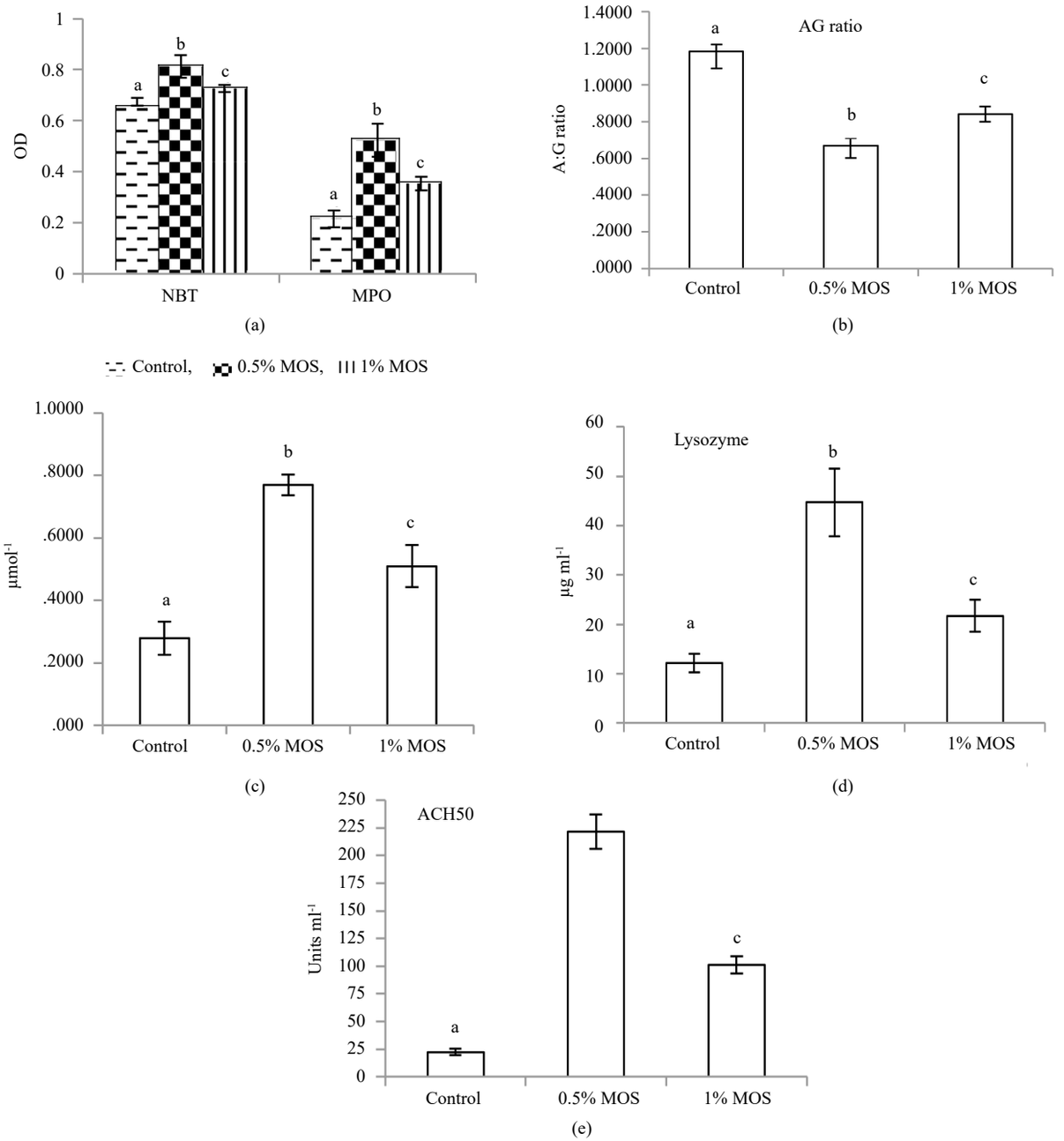

Fig. 3. Innate immune parameters in control and MOS fed experimental groups. (a) RBA and MPO activity, (b) A/G ratio, (c) NO production, (d) Lysozyme activity and ACH50 titre. Superscripts with different alphabets denote significant difference ($p<0.05$, Mean±SD). OD: Optical density; NBT: Nitroblue tetrazolium; MPO: Myeloperoxidase; A:G: Albumin/Globulin; ACH50: Alternate complement hemolytic activity; NO: Nitric oxide; MOS: Mannan oligosaccharide

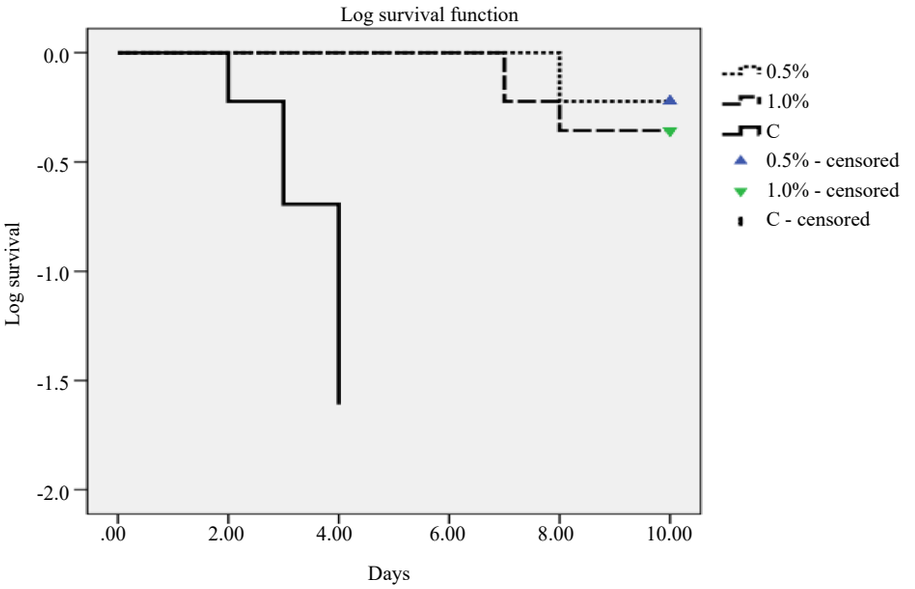

Fig. 4. Relative percentage survival (RPS) against virulent *A. hydrophila* challenge between treatments. (Survival rate analysis was done by Kaplan-Meier method in SPSS software)

weight gain after feeding MOS incorporated diets, with no difference ($p>0.05$) between 0.5 and 1% levels. Another carp, *Labeo rohita* fed with MOS at 1, 2 and 4% dietary levels also recorded higher growth compared to Control, with the highest growth in 1% followed by 2 and 4%, indicating levels higher than 1% may not be beneficial (Andrews *et al.,* 2009). Several authors have reported improved growth performance of fish fed MOS supplemented diets (Staykov *et al.,* 2007; Wu *et al.,* 2014; Akter *et al.,* 2016; Ali *et al.,* 2017). MOS at lower levels *viz,* 0.15, 0.3 and 0.45% were not effective ($p>0.05$) in promoting growth and feed conversion in Gibel carp *Carassius auratus gibelio* (Akrami *et al.,* 2012), but a supplementation level of 0.2% in rainbow trout diets significantly increased growth and feed efficiency (Staykov *et al.,* 2007). Hanley *et al.* (1995) reported an improved performance of red tilapia fed 0.6% MOS and Zhou and Li (2004) reported improved growth and food conversion in Jian carp fed MOS at 0.24%. However, no improvement in growth performance and feed efficiency by dietary MOS was reported in other species like African catfish (Genc *et al.,* 2006), hybrid tilapia (Genc *et al.,* 2007), European seabass (Torrecillas *et al.,* 2007), pacu (Sado *et al.,* 2014) and Nile tilapia (Sado *et al.,* 2017) indicating variations according to differences in MOS inclusion level and culture conditions as well as species specific differences.

The feed conversion was higher ($p<0.05$) with MOS incorporated diets, with the lowest FCR recorded at 0.5% MOS incorporation. Better feed conversion was also recorded by Andrews *et al.* (2009) in rohu fed MOS incorporated diets. Our results showed that the growth performance of *B. carnaticus* ($p>0.05$), FCR ($p<0.05$) and PER ($p<0.05$) tended to decrease as the dietary supplementation of MOS increased to 1% level. Similar results with MOS were also recorded by earlier researchers (Daniels *et al.,* 2006; Andrews *et al.,* 2009). The 'Condition factor', which is an index of the well-being of the fish under culture was higher for fish fed MOS. Ali *et al.* (2017) also recorded similar findings in Asian seabass fed MOS at 1-2% levels. Andrews *et al.* (2009) recorded higher survival of rohu fed MOS at 1, 2 and 4% dietary levels and rainbow trout fed MOS at 0.2%. However, in the present study, 100% survival was recorded in all groups, probably owing to the higher initial weight of fish (around 21 g).

The positive effects of MOS on growth may be associated with an alteration of the intestinal microflora and an improvement of nutrient digestibility (Gultepe *et al.,* 2010). MOS supplementation significantly improved nutrient digestion in gilthead seabream (Gultepe *et al.,* 2010). The higher activity of hepatopancreatic ($p<0.05$) and intestinal ($p>0.05$) protease, lipase and amylase in *B. carnaticus* fed 0.5% MOS corroborates these observations. The activity showed a decrease at 1% level of incorporation. Similarly, higher amylase, protease and lipase activities were recorded by Akter *et al.* (2016) in striped catfish fed 0.6% MOS, with a decrease at still higher MOS levels. These findings suggest that the increase in the activity of digestive enzymes has an intrinsic limit. The reduction in enzyme activity may be attributed to the inability of intestinal microflora to ferment excessive levels

of prebiotics and subsequent accumulation in the intestine which may be deleterious to the enterocytes (Soleimani *et al.,* 2012). Zhou and Li (2004) and Wu *et al.* (2014) also reported improvement in digestive enzyme activity in fish post-feeding MOS. Improved digestive enzyme activity in fish with prebiotics diets feeding has been reported in several studies (Xu *et al.,* 2009; Sang *et al.,* 2011). Earlier studies have attributed the improved growth of MOS fed fish to the improved nutrient utilisation due to increased microvilli density in the intestine (Dimitroglou *et al.,* 2010; Torrecillas *et al.,* 2011; Ali *et al.,* 2015). Andrews *et al.* (2009) attributed the higher digestive enzyme activity to the production of extracellular enzymes by the gut microflora. Further, MOS is known to promote the growth of lactic acid bacteria in the intestine, since it is used as an energy source by them (Miles, 1993).

Carcass proximate composition analysis of *B. carnaticus* revealed no difference in the moisture, fat and ash contents. However, the crude protein content was higher with MOS treated fish. In rainbow trout and hybrid tilapia also, the body concentration of protein has been reported to be increased by feeding MOS. Studies conducted by Dimitroglou *et al.* (2010) and Gultepe *et al.* (2011) on gilthead seabream, Akrami *et al.* (2012) on Kutum, Razeghi *et al.* (2012) on giant sturgeon and Ali *et al.* (2015) on Asian seabass, on the other hand, revealed no difference ($p<0.05$) in carcass composition between control fish and those fed MOS incorporated diets.

MOS is a proven prebiotic for its immune modulatory effect when included in the diets of various fish species (Torrecells *et al.,* 2014). In the present study, inclusion of MOS at 0.5% in the diets enhanced the innate immune functions as measured through respiratory burst, myeloperoxidase activity, nitric oxide and lysozyme activity. Respiratory burst is a physiological process of generating reactive oxygen species by phagocytic cells for killing of pathogens (Sahoo *et. al.,* 2005; Selim *et al.,* 2015). In the present study, the respiratory burst was significantly ($p<0.05$) higher in MOS fed diet and these results are in agreement with earlier reports which describe increased respiratory burst in different fish species fed with MOS diet **(**Rodriguez *et al.,* 2003; Torrecillas *et al.*, 2007, 2011; Liu *et al.,* 2013; Selim *et al.,* 2015). Myeloperoxidase enzyme plays an important role in regulating formation of neutrophil extracellular traps and production of reactive intermediate species which are microbicidal in nature (Yeh and Klesius, 2013). Inclusion of MOS at different concentrations in the diet of different fish species has elevated the serum MPO levels as reported by previous research groups (Rodriguez *et al.,* 2003; Torrecillas *et al.,* 2007; Andrews *et al.,* 2009; Buentello *et al.,* 2010; Peterson *et al.,* 2010; Sang *et al.,* 2011; Torrecillas *et al.,* 2011; Welker *et al.,* 2012; Liu *et al.*, 2013) which is in agreement with results of the present study. Lysozyme is a robust anti-bactericidal enzyme synthesised by phagocytic cells of the body as a defensive mechanism against pathogens. Many research groups have reported that MOS inclusion in the diet significantly elevated serum lysozyme activity in different fish species (Torrecillas *et al.,* 2007, 2011, 2012; Liu *et al.,* 2013; Selim *et al.,* 2015; Lee *et al.,* 2018). In the presnet study, inclusion of MOS at 0.5 and 1% in the diet has significantly ($p<0.05$) enhanced the serum lysozyme levels which is in agreement with earlier reports. Nitric oxide is an important microbicidal molecule which is produced by phagocytic cells in response to antigen, adjuvants and pathogen stimulation (Das *et al*., 2018). Selim *et al.* (2015) reported that dietary MOS significantly enhanced the serum NO levels in Nile tilapia as recorded in the present study.

A/G ratio is an important indicator of robustness of the host resistance and immunity and the results in the present study indicate the effect of MOS in enhanced globular fraction in the serum protein. The results are in accordance with earlier reports which described the role of MOS in increasing the globulin levels in the serum (Torrecillas *et al.,* 2007; Andrews *et al.,* 2009). The globulin fraction mainly includes immuoglobulins and in fish it is mainly IgM. The increased level of globulin may be due to MOS activation of T-cells which in turn trigger B-cell activation and immunoglobulin production (Torrecellas *et al.,* 2015).

Complement cascade plays an important role in the process of killing and elimination of pathogen from the host. In the present study, inclusion of MOS in fish diet has shown enhanced ACH50 activity and possible role of MOS in activation of complement cascade. These results are in agreement with the earlier reports which indicate role of MOS in enhanced complement activity in different fish species (Stayon *et al.,* 2007; Torrecillas *et al.,* 2007). The enhanced ACH50 may be due to triggering of mannose binding lectin (MBL) in liver secretions by MOS and MBL in turn activates complement cascade to eliminate the pathogens (Torrecillas *et al.,* 2007).

Further, mannose sugar is an important component of MOS structure which interacts with lectins and other receptors like pattern recognition receptors (PPRs) present in the gut enterocytes and immune cells (Torrecillas *et al.,* 2011). This interaction stimulates various downstream signaling pathways which help in activation of inflammatory immune cells. Activated immune cells secrete microbicidal agents and molecules which enhance cellular respiratory burst, myeloperoxidase activity, nitric oxide and lysozyme activity and help in killing

of pathogens and provide resistance to host against pathogens.

The decreased innate immune functions in the group fed with 1% MOS than 0.5% MOS may be due to the tolerance of immune cells for higher concentration of the prebiotic. The dose for this immune tolerance may vary with different fish species. These results are in accordance with earlier reports which states the higher concentration of MOS has immune suppression effect (Stayon *et al.,* 2007; Torrecillas *et al.,* 2007, 2015; Andrews *et al.,* 2009). The higher relative percentage of survival (RPS) in fish group fed with 0.5% MOS may be due to stimulation of innate immune defense mechanism by MOS which helped in preventing pathogen multiplication and spread within the host. These results are in agreement with earlier published reports which describe the role of MOS in protecting fish against various fish pathogens (Andrews *et al.,* 2009; Torrecillas *et al.,* 2012; 2014; Liu *et al.,* 2013; Lee *et al.,* 2018).

The study revealed that MOS supplementation improves the digestive enzyme activity and has got beneficial effect on the growth of *B. carnaticus*. Inclusion of MOS at 0.5% would be beneficial in terms of potentiating host innate immune responses and protecting the fish against bacterial fish pathogens.

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