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Effect of dietary fructooligosaccharide supplementation on growth, body composition, hematological and immunological parameters of Asian seabass (*Lates calcarifer*)

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Abstract A feeding trial to study the effect of addition of prebiotic fructooligosaccharide (FOS) on feeds for juvenile Asian seabass (*Lates calcarifer*) (12.2 ± 0.4 g) was carried out. Five isonitrogenous and isocaloric experimental diets were formulated that contained 400 g kg^{-1} protein and 90 g kg^{-1} lipid. The FOS was supplemented at 2.5, 5, 7.5 and 10 g kg^{-1} in the diet of *L. calcarifer* with a control that was devoid of FOS. The trial was carried out in 1000-L fiber reinforced polymer (FRP) tanks with three replicates (each containing 20 fish) for each treatment. After 45 days of feeding, it was observed that FOS supplementation at 10 g kg^{-1} in the diet resulted in significantly ($P < 0.05$) higher final biomass (334.2 ± 7.3 g) and survival (97.7 ± 3.8 %). There were no significant ($P > 0.05$) differences in biological indices of fish fed with the experimental and control diets. Whole body chemical composition of animals post-feeding revealed significantly ($P < 0.05$) higher crude protein, crude lipid and total ash content in 5 g kg^{-1} FOS-supplemented diet. The analysis of hematological parameters revealed that red blood cells (RBC), white blood cells, hemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration levels increased significantly ($P < 0.05$) with dietary FOS supplementation up to 5 g kg^{-1}.The second-degree polynomial regression analysis of RBC (4.06 ± 0.05 × 10^6 mm^{-3}) showed that FOS 5 g kg^{-1} is an optimal level in seabass diet. Significantly ($P < 0.05$) higher lysozyme (77.4 ± 0.5 U mL^{-1}) and superoxide dismutase (75.2 ± 0.3 U mL^{-1}) activity was recorded at 10 g kg^{-1} FOS supplementation. It could therefore be concluded that 10 g kg^{-1} FOS supplementation has a beneficial effect in improving the survival rate and immunological parameters in *L. calcarifer* juveniles.

Keywords Barramundi · Biological indices · Feed additive · Prebiotics · Survival

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Introduction

The Asian seabass (*Lates calcarifer*), also known as barramundi, is a euryhaline carnivorous fish with a wide distribution in the tropical and subtropical regions of the western Pacific and Indian Oceans (Newton et al. 2010). Currently, this fish is increasingly being seen as an emerging species in global aquaculture and is being considered as an alternate candidate species in brackishwater aquaculture in India (FAO 2014). In large-scale aquaculture production including seabass, the aquatic animals are exposed to stressful conditions and often leading to disease susceptibility accompanied with mortalities resulting in serious economic losses (Anh et al. 2010). Current methods for prevention and treatment of infectious aquatic diseases include vaccines, antibiotics and chemotherapeutics (Rico and Van den Brink 2014). However, antibiotics usage has been extensively criticized for potential development of antibiotic-resistant bacterial strains and destruction of environmental microbial flora, as well as having relatively high cost and marginal effects in some cases (Rico et al. 2012). Certain antibiotics have been shown to suppress the immune system potentially making aquaculture organisms more susceptible to viral or parasitic infections (Sapkota et al. 2008). Prebiotics have recently attracted extensive attention in aquaculture because of its natural origin and less influence on natural environment (Ringo et al. 2010). However, prebiotics are often used as a prophylactic strategy rather than curative, thereby reducing the need for antibiotics (Talpur et al. 2014). Prebiotics are a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Manning and Gibson 2004).

Dietary supplementation of fructooligosaccharide (FOS) has been shown to enhance growth rates and/or the survival of aquatic animals (Soleimani et al. 2012; Akrami et al. 2013; Ortiz et al. 2013; Safari et al. 2014). Our earlier studies using prebiotic, mannanoligosaccharide (MOS) and inulin showed beneficial effect in improving the health and growth of seabass (Syed Raffic Ali et al. 2015, 2016). However, the effect of dietary prebiotic, FOS in the tropical fish species, Asian seabass has not been attempted so far. Therefore, the present study was carried out to assess the effect of FOS supplementation as a dietary prebiotic on growth, body composition, hematological and immunological parameters of *L. calcarifer* juveniles.

Materials and methods

Preparation of experimental diets

The effect of a dietary supplementation of FOS was studied by adding 2.5, 5, 7.5 and 10 g FOS kg^{-1} to a control (400 g kg^{-1} protein; 90 g kg^{-1} lipid) seabass diet (Table 1) (CIBA Bhetkiahar).

Dry solid feed ingredients were powdered in an electrical grinder and allowed to pass through a 0.5-mm sieve. They were mixed with additives and homogenized thoroughly in an electrical blender. The diet mix was made into soft dough using required quantity of water and was steam-cooked (at atmospheric pressure) for 5 min, cooled and hand-pelletized using a 2.0-mm die. After oven drying at 50 °C, the feed samples were packed in air-tight containers and stored at −20 °C for feeding.

Table 1 Ingredients and proximate composition of experimental diets

Diets	Control	FOS 2.5	FOS 5	FOS 7.5	FOS 10
Ingredients (g kg^{-1})					
Fish meal[a]	400	400	400	400	400
Soybean meal	250	250	250	250	250
Wheat	140	140	140	140	140
Rice	50	50	50	50	50
Maize	50	50	50	50	50
Fish oil[a]	40	40	40	40	40
Lecithin	20	20	20	20	20
Vitamin and mineral[b]	30	30	30	30	30
Binder[c]	10	10	10	10	10
Cellulose	10	7.5	5	2.5	0
FOS[d]	0	2.5	5	7.5	10
Proximate composition (g kg^{-1})					
Moisture	87.5	82.6	84.4	82.0	82.4
Crude protein	403.2	403.7	403.6	403.2	404.6
Crude lipid	88.3	88.2	88.5	88.1	88.4
Crude fiber	20.6	21.2	22.7	21.2	21.5
Total ash	141.8	140.1	144.7	141.2	131.0
Nitrogen-free extract	258.6	264.2	256.1	264.3	272.1

[a] Sardine fishmeal and fish oil. Bismi Fisheries, Mayiladuthurai, Tamil Nadu, India

[b] Commercially sourced premix and each kg contains vitamin A—2000000 IU, vitamin D—400000 IU, vitamin E—300 U, vitamin K—450 mg, riboflavin—800 mg, panthothenic acid—1 g, nicotinamide—4 g, witamin B12—2.4 mg, choline chloride—60 g, Ca—300 g, Mg—11 g, I—400 mg, Fe—3 g, Zn—6 g, Cu—800 mg, Co—180 mg, Sarabhai Zydus Animal Health Ltd, Vadodara, Gujarat, India

[c] Pegabind, Bentoli AgriNutrition Asia Pvt Ltd, Singapore

[d] FOS—Himedia

Fish rearing and experimental design

Hatchery bred and farm-reared Asian seabass juveniles were procured from a farm at Pulicat, 60 KM north of Chennai, India, and transported to the nutrition wet laboratory at the Muttukadu Experimental Station of CIBA. They were acclimatized for a fortnight and fed with the control diet (CIBA Bhetkiahar).The juveniles (average body weight: 12.2 ± 0.4 g) were randomly distributed into fifteen 1000-L oval FRP tanks. A completely randomized design was used with three replicates (20 animals each) for each treatment. The tanks were supplied with sand-filtered seawater with provisions for continuous aeration through air diffuser stones. Throughout the trial, water in the tanks was exchanged once in the morning and evening. Fishes were hand-fed in excess twice daily (10.00 and 16.00 h). After 30 min of the feeding, unconsumed feed was siphoned out and dried to determine the actual feed consumption. Animals were weighed individually at the start and end of the experiment, whereas bulk weighing was carried out fortnightly to monitor growth. Fish were maintained under a natural photoperiodicity (12-h L: 12-h D).The water quality parameters were measured once a week by standard methods (APHA 1998), viz. temperature 26–29 °C; salinity 28–31 ‰, pH 7.4–8.2; dissolved oxygen 6.0–7.3 mg L^{-1}; and total ammonia nitrogen 0.08–0.11 mg $L^{-1.}$

Chemical analysis

The proximate composition of the ingredients, experimental diets and whole body composition of fish were analyzed by standard procedures as per (AOAC 2012). At the termination of the experiment, 4 fish from each tank were collected and killed by over dose of anesthesia for determination of whole body composition. The fish samples were homogenized and dried at 105 °C for 24 h. The dried samples within a tank were pooled and analyzed.

Moisture content was estimated by gravimetric analysis after oven drying at 105 °C for 12 h. Crude protein (CP) was determined by Kjeldahl method (N × 6.25) after acid hydrolysis (Kjeltec 2100, FOSS, Tecator, Sweden). Crude lipid (CL) was calculated gravimetrically after extraction with petroleum ether in a soxhlet system SOCS, Pelican, India. Total ash was determined gravimetrically by ignition at 600 °C for 6 h in muffle furnace. Crude fiber was estimated gravimetrically after acid and alkali digestion and loss in mass by combustion at 600 °C for 3 h. Nitrogen-free extract (NFE) was calculated from 1000 − (crude protein + crude lipid + crude fiber + total ash). All the chemical analyses were carried out in triplicate, and the results were expressed in wet weight basis.

Blood sample collection

At the end of the experiment, three fishes were randomly chosen from each tank and anaesthetized using 2-phenoxyethanol at a dose of 0.3 mL L^{-1}. About 1 mL of blood was drawn from the caudal vein, using a 2-mL syringe with 26-G needle. One half of the blood sample was transferred to heparinized tubes while the other portion was transferred to non-heparinized tubes to analyze hematological parameters and immunological assay, respectively. For serum separation, blood samples in the non-heparinized tubes were centrifuged at 1000 rpm for 5 min to separate the serum. Serum samples were preserved at −20 °C until use (Jalali et al. 2009).

Hematological parameters

The heparinized blood samples were used for analysis of hematological parameters. Red blood cell count (RBC) and white blood cell count (WBC) were determined using a Neubauer hemocytometer after Blaxhall and Daisley (1973). The packed cell volume (PCV) was measured using standard microhematocrit method and reported as percentages as described by England and Walford (1972). Hemoglobin (Hb) levels were estimated by cyanomethemoglobin method after Blaxhall and Daisley (1973). The erythrocytes indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated after Blaxhall and Daisley (1973).

Immunological assays

Lysozyme activity

Serum lysozyme activity was determined by turbidimetric assay according to the method described by Ellis (1990). Briefly, test serum (0.1 mL) was added to 1.9 mL of a suspension of *Micrococcus lysodeikticus* (Sigma) (0.2 mg mL^{-1}) in a 0.05 M phosphate-

buffered saline (PBS) (pH 6.2). The reaction was carried out at 25 °C, and absorbance was measured at 530 nm after 0.5 and 4.5 min in a spectrophotometer. One unit of lysozyme activity was defined as the amount of sample causing a reduction in absorbance of 0.001 $min^{-1.}$

Superoxide dismutase activity

Superoxide dismutase activity (SOD) assay was determined according to the method described by Misra and Fridovich (1972). Briefly 0.1 mL of sample and 0.75 mL of ethanol and 0.15 mL of chloroform (chilled in ice) were added and centrifuged at 10,000 rpm at 4 °C for 10 min. To 0.5 mL of the supernatant, 0.5 mL of EDTA (0.6 mM) solution and 1 mL of carbonate bicarbonate buffer (0.1 M, pH 10.2) were added. The reaction was initiated by the addition of 0.5 mL of substrate (epinephrine 1.8 mM), and the increase in absorbance was recorded at 480 nm every 30 s for 3 min.

Data recording

On termination of the experiment, fish were anaesthetized using 2-phenoxyethanol at a dose of 0.3 mL L^{-1} and the total length and weight of each fish recorded. Three fish from each tank were randomly selected to measure the biometric indices. Liver and viscera of fish were dissected out and weighed for computation of hepatosomatic index (HSI) and viscerosomatic index (VSI) (Nandakumar et al. 2013). Growth parameters were calculated as detailed below.

IBW (g) = initial body weight
FBW (g) = final body weight
Survival (%) = (final count of fish/initial count of fish) × 100
Feed conversion ratio (FCR) = feed consumed (g, dry weight)/weight gain (g)
Condition factor (CF, g $(cm^3)^{-1}$) = [(live weight, g)/(length, cm)^3] × 100
Hepatosomatic index (HSI, %) = (liver weight, g/body weight, g) × 100
Viscerosomatic index (VSI, %) = (visceral weight, g/body weight, g) × 100

Statistical analysis

Data were analyzed using ANOVA to compare significant differences between treatments. Significance of treatments was tested by Duncan's multiple range test. The second-degree polynomial regression analysis was carried out on RBC values to find out the optimal level of FOS. All data were analyzed using SPSS version 16.0 software (SPSS, Chicago, IL, USA).

Results

Growth performance and survival

The growth performance and survival of seabass fed with FOS-supplemented diets are depicted in Table 2. The results showed that there were no significant ($P > 0.05$) differences in final body weight (FBW) and feed conversion ratio (FCR) among different

Table 2 Growth performance and survival of Asian seabass fed experimental diets supplemented with varying levels of FOS for 45 days

Parameters	Control	FOS 2.5	FOS 5	FOS 7.5	FOS 10
IBW (g)	12.2 ± 0.4	11.8 ± 0.5	11.6 ± 0.3	11.6 ± 0.1	11.5 ± 0.3
FBW (g)	23.7 ± 0.7	23.5 ± 0.3	22.8 ± 0.5	23.2 ± 0.7	22.7 ± 1.0
Final biomass (g)	277.2^{a} ± 9.3	290.1^{a} ± 8.3	274.9^{a} ± 15.4	278.8^{a} ± 5.0	334.2^{b} ± 7.3
Survival (%)	77.7^{a} ± 3.8	82.2^{a} ± 3.8	80.0^{a} ± 6.6	80.0^{a} ± 0.0	97.7^{b} ± 3.8
FCR	1.49 ± 0.06	1.49 ± 0.04	1.47 ± 0.05	1.49 ± 0.04	1.52 ± 0.03

All values are mean ± SE of three observations

Mean bearing different superscript in a row differs significantly ($P < 0.05$)

Table 3 Whole body composition (% wet weight) of Asian seabass fed experimental diets supplemented with varying levels of FOS for 45 days

Parameters	Control	FOS 2.5	FOS 5	FOS 7.5	FOS 10
Moisture	70.0 ± 0.35	69.8 ± 0.98	68.4 ± 1.51	68.1 ± 2.05	68.0 ± 1.94
Crude protein	17.4^{ab} ± 1.19	17.4^{ab} ± 0.05	19.1^{c} ± 0.33	16.6^{a} ± 0.04	17.8^{b} ± 0.07
Crude lipid	2.75^{a} ± 0.19	3.05^{b} ± 0.01	4.17^{e} ± 0.10	3.31^{c} ± 0.03	3.55^{d} ± 0.02
Total ash	7.50^{ab} ± 0.49	7.68^{b} ± 0.20	8.15^{c} ± 0.14	7.10^{a} ± 0.01	7.72^{b} ± 0.02

All values are mean ± SE of three observations

Mean bearing different superscript in a row differ significantly ($P < 0.05$)

treatments, while significantly ($P < 0.05$) higher final biomass and survival was observed in groups fed with diet containing 10 g kg^{-1} FOS compared to other treatments.

Whole body composition and biological indices

Whole body composition of seabass fed with FOS-supplemented diets is presented in Table 3. Analysis of moisture content of seabass fed with experimental diets showed no significant differences ($P > 0.05$) among the treatment groups. Crude protein, crude lipid and total ash content was significantly ($P < 0.05$) higher in fish fed with 5 g kg^{-1} FOS diet. The biological indices of seabass fed with FOS-supplemented diets are presented in Table 4. Condition factor (CF), hepatosomatic index (HSI) and viscerosomatic index (VSI) of post-fed experimental animals showed no significant ($P > 0.05$) differences among the treatment groups.

Hematological and immunological parameters

Hematological parameters of seabass fed with FOS-supplemented diets are presented in Table 5. The results showed that RBC, WBC, Hb, PCV, MCV, MCH and MCHC levels increased significantly ($P < 0.05$) in the group fed with 5 g kg^{-1} FOS. The results revealed that 5 g kg^{-1} FOS supplementation has a positive effect on RBC, WBC and Hb. The second-degree polynomial regression analysis of red blood cells (RBC) with varying levels FOS is shown in (Fig. 1). Immunological assays of seabass fed with FOS-

Table 4 Biological indices of Asian seabass fed experimental diets supplemented with varying levels of FOS for 45 days

Parameters	Control	FOS 2.5	FOS 5	FOS 7.5	FOS 10
CF (k)	1.14 ± 0.01	1.12 ± 0.02	1.16 ± 0.02	1.14 ± 0.04	1.15 ± 0.02
HSI (%)	1.47 ± 0.03	1.44 ± 0.03	1.48 ± 0.02	1.46 ± 0.02	1.48 ± 0.05
VSI (%)	7.27 ± 0.04	7.31 ± 0.11	7.33 ± 0.09	7.40 ± 0.08	7.38 ± 0.07

All values are mean ± SE of three observations

No significant differences for all parameters ($P > 0.05$)

Table 5 Hematological parameters of Asian seabass fed experimental diets supplemented with varying levels of FOS for 45 days

Parameters	Control	FOS 2.5	FOS 5	FOS 7.5	FOS 10
RBC (10^6 mm^{-3})	3.14^a ± 0.03	3.40^b ± 0.03	4.06^d ± 0.05	3.64^c ± 0.03	3.68^c ± 0.05
WBC (10^3 mm^{-3})	7.33^a ± 0.03	7.63^b ± 0.04	8.17^e ± 0.06	7.90^d ± 0.02	7.72^c ± 0.05
Hb (g L^{-1})	74.3^a ± 1.5	81.3^b ± 2.0	87.3^d ± 0.5	85.3^c ± 2.0	80.6^b ± 1.5
PCV (%)	38.5^a ± 0.4	38.3^a ± 0.2	39.3^b ± 0.4	38.3^a ± 0.1	38.3^a ± 0.2
MCV(fl)	96.3^a ± 0.2	97.3^b ± 0.1	99.4^d ± 0.1	98.2^c ± 0.1	97.2^b ± 0.3
MCH (pg)	21.4^a ± 0.2	21.7^b ± 0.1	23.2^c ± 0.1	21.3^a ± 0.0	23.3^c ± 0.1
MCHC (g L^{-1})	213^a ± 0.5	215^a ± 1.0	222^b ± 1.5	231^e ± 1.7	225^d ± 2.0

All values are mean ± SE of three observations

Mean bearing different superscript in a row differ significantly ($P < 0.05$)

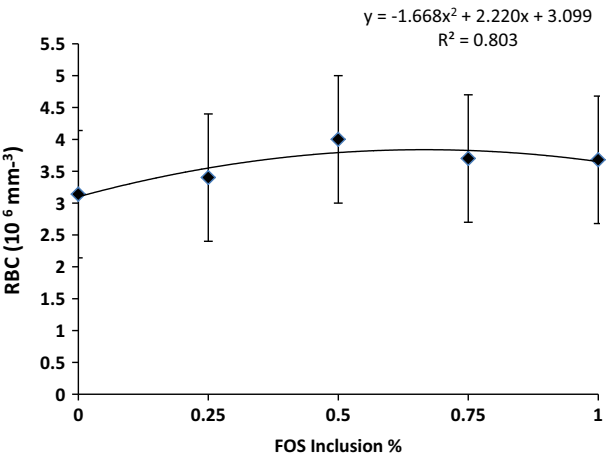

Fig. 1 Second-degree polynomial regression analysis of red blood cells (RBC) with varying levels of FOS

supplemented diets are shown in (Figs. 2, 3). The lysozyme and SOD activity of seabass fed with FOS-supplemented diet showed significant ($P < 0.05$) differences among the treatment groups compared to the control.

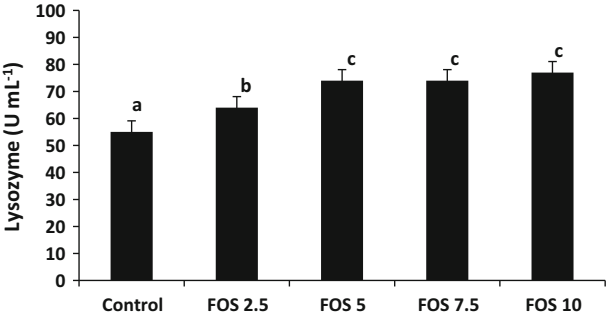

Fig. 2 Lysozyme activity of Asian seabass fed experimental diets with varying levels of FOS for 45 days. *Bars* assigned with different superscripts are significantly different ($P < 0.05$)

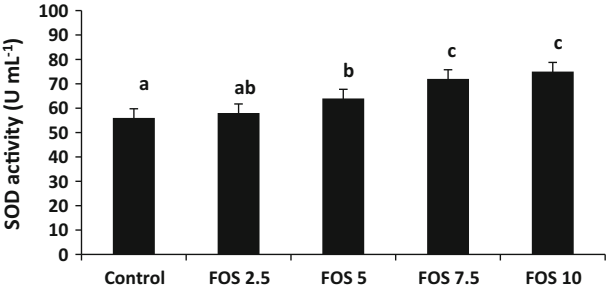

Fig. 3 Superoxide dismutase activity (SOD) of Asian seabass fed experimental diets with varying levels of FOS for 45 days. *Bars* assigned with different superscripts are significantly different ($P < 0.05$)

Discussion

This is the first study to investigate the effect of FOS on growth, body composition, hematology and immunological parameters in *L. calcarifer*. The results revealed that there was no significant effect of FOS supplementation on final body weight and FCR while significantly higher final biomass and survival was observed in the diet supplemented with 10 g kg^{-1} FOS compared to other treatments. Our results are in agreement with those reported by He et al. (2003) and Hoseinifar et al. (2011) who reported no significant effect of FOS on growth performance. On the contrary, improved growth performance was reported by Soleimani et al. (2012), Akrami et al. (2013), Ortiz et al. (2013) and Safari et al. (2014). The role of prebiotics in enhancing the immune system has been extensively studied in aquatic animals (Torrecillas et al. 2007). Adding FOS to culture systems has been shown to reduce the growth of potentially pathogenic organisms and increase the number of bifidobacteria and lactic acid bacteria (Gibson and Wang 1994). Dietary supplementation of FOS recently has been shown to enhance growth rate of aquatic animals (Zhang et al. 2010). In the present study, dietary supplementation of FOS (0.25–10 g kg^{-1}) improved only the survival and did not extend beneficial effects on growth. The health-promoting effect of dietary FOS has been reported in grass carp (*Ctenopharyngodon idellus*) (Li et al. 2012) and Allogynogenetic crucian carp (*Carassius auratus gibelio*) (Liu et al. 2013). The variation in growth, feed utilization and health benefits with the dietary

use of prebiotics is dependent on fish species, feeding duration as well as the type of prebiotics (Taati et al. 2011).

The whole body composition of seabass fed with FOS-supplemented diets showed significant variations in crude protein, crude lipid and total ash content, and the group fed with 5 g kg^{-1} FOS showed significantly higher crude protein, crude lipid and total ash content than compared to the other treatments. Wu et al. (2013) reported that FOS supplementation at 2 and 4 g kg^{-1} resulted in significantly higher lipid content in the whole body of blunt snout bream (*Megalobrama amblycephala*) fingerlings. On the contrary, Grisdale-Helland et al. (2008) observed no significant differences in Atlantic salmon (*Salmo salar*). The condition factor, HSI and VSI showed non-significant differences between control and FOS-supplemented groups. Condition factor is used to compare the 'condition,' 'fatness' or 'well being' of fish and is based on the hypothesis that heavier fish of a given length are in better condition. Zhou et al. (2010) had reported nonsignificant differences in CF and HSI in red drum (*Sciaenops ocellatus*) fed diets containing different prebiotics like FOS, MOS, GOS and Previda at 10 g kg^{-1} inclusion level. Hepatosomatic index is directly related to metabolism because glycogen and lipids can be stored in the liver (Dimitroglou et al. 2010).

The results of the hematological parameters showed that RBC, WBC, Hb, PCV, MCV MCH and MCHC increased significantly in fish fed with 5 g kg^{-1} FOS. Akrami et al. (2013) reported that RBC, Hb, PCV, MCV and lymphocyte increased in the fish treated with 10 g kg^{-1} FOS. Hoseinifar et al. (2010) reported that WBC levels, particularly lymphocytes, were significantly increased in beluga fed with 1 and 2 g kg^{-1} dietary oligofructose. The elevated leukocyte levels could have been responsible for the increase in the activity-enhancing defense mechanism during feeding. Leukocytes are important cells that can stimulate immune responses in fish. These cells produce antibodies and can perform macrophages activities (Jalali et al. 2009). In fish, blood parameters like RBC, WBC and hemoglobin are frequently used as indicators of health status and are involved in regulation of immunological function in the organism. Blaxhall and Daisley (1973) have reported the possibility of using PCV as a tool in aquaculture for checking anemic condition. Reported values for fish PCV are usually between 20 and 35 % and scarcely attain values greater than 50 %. The MCV, MCH and MCHC have a particular importance in the diagnosis of anemia in most animals. The hematological parameters of fish are reported to be affected by a range of factors, which include species, size, age, physiological status, environmental conditions, dietary regime, quality and quantity of food, dietary ingredients, protein sources, vitamins and probiotics (Osuigwe et al. 2005).

The serum lysozyme activity increased significantly in fish fed with FOS 10 g kg^{-1} diet compared to the other treatments. Zhou et al. 2010; Soleimani et al. 2012; Zhang et al. 2014 have also reported that FOS-supplemented diets had significantly greater serum lysozyme activity compared to the control. In contrast, no significant effects were observed on serum lysozyme activity in Atlantic salmon fed with FOS-supplemented diet compared to the control diet (Grisdale-Helland et al. 2008).The innate immune system in aquatic animals is continually vulnerable to numerous opportunistic pathogens, and this part of immune response provides the first line of defense for the host (Soleimani et al. 2012). Lysozyme is one of the important defense elements against parasitic, bacterial and viral infections, which plays an important role as an opsonin to facilitate the lysis of pathogens by making them prone to phagocytosis. The SOD activity increased significantly in fish fed with the diet supplemented with 10 g kg^{-1} FOS.

Similar result was reported in grass carp (*Ctenopharyngodon idellus*) (Li et al. 2012) and shrimp (*Litopenaeus vannamei*) (Shen et al. 2010). Supplementation of FOS at

0.3 g kg^{-1} resulted in increased antioxidant capacity in triangular bream (*Megalobrama terminalis*) (Zhang et al. 2013). As the first line of antioxidant enzymatic defense, SOD is one of the important biochemical parameters for antioxidant defense (Li et al. 2012).

The results of this study revealed that FOS supplementation in the diet of Asian seabass had a beneficial effect on improving survival, hematological and immunological parameters. It may be inferred that supplementation of FOS at 10 g kg^{-1} in the diet is beneficial. However, further studies are warranted to ascertain the prebiotic effect of FOS and to conclusively optimize the level of supplementation in practical diets as an immune enhancer.

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