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Effect of dietary prebiotic inulin on histology, immuno-haematological and biochemical parameters of Asian seabass (*Lates calcarifer*)

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Abstract

The present study was carried out to investigate the effect of supplementation of inulin on histology, immuno-haematological and biochemical parameters of Asian seabass (*Lates calcarifer*) fingerlings with an initial average body weight of 7.14 ± 0.05 g. Inulin was supplemented at four different concentrations (control) 0, 5, 10, 15, and 20 g/kg in the *L. calcarifer* diet containing 400 g/kg protein and 90 g/kg lipid. At the end of the 60 days feeding trial, the absorptive surface area of the intestine and glycogen deposition in liver were increased in fish fed inulin supplemented diets. The immune parameters lysozyme, alternative complement pathway, superoxide dismutase and nitroblue tetrazolium assay showed significant ($p < 0.05$) difference between control and treatment groups. Haematological parameters showed that red blood cells, white blood cells, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration levels increased significantly ($p < 0.05$) in the fish fed with inulin supplemented diets. The analysis of biochemical parameters revealed that glucose, urea, cholesterol, triglyceride, aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase, and lactate dehydrogenase showed significant differences ($p < 0.05$) between control and treatments groups. No significant difference ($p > 0.05$) was observed for total protein among different treatments. The results of the study revealed that 15–20 g/kg inulin supplementation has a beneficial effect in the histology, immuno-haematological, and biochemical parameters in *L. calcarifer* juveniles.

KEYWORDS
barramundi, blood parameters, immunological parameters, intestine, inulin, liver

1 | INTRODUCTION

Asian seabass (*Lates calcarifer*) commonly known as barramundi, is an economically important species and it is widely cultured in many Asian countries in fresh, brackish, and marine water resource (Syed Raffic Ali, Ambasankar, Musthafa, & Harikrishnan, 2017). 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 production of this species under the crowded condition, is exposed to stress often leading to disease susceptibility accompanied with mass mortalities resulting in serious economic losses due to diseases and the loss caused by pathogens is estimated to be around 10%–20% of farmed fish (Newaj-Fyzul & Austin, 2015). Bacterial and viral

infections often affect cultured aquatic organisms, whose immune system may be compromised by stressful conditions (Jitender Kumar, Satayanarayana, & Mukesh Kumar, 2011). More than three decades, antibiotic agents were routinely administered for control of bacterial diseases in commercial aquaculture (Faggio, Fazio, Marafioti, Arfuso, & Piccione, 2015). However, as the abuse of antibiotics lead to emergence antibiotic resistance and immune system depression, necessitating the scientific community to search for alternative control strategies such as administration of supplements to substitute the therapeutic use of antimicrobials (Hoseinifar, Ringo, Shenavar, & Esteban, 2016). One of the alternatives to antibiotics is the use of prebiotics, and its administration has been suggested as promising strategies for modulation of the fish immune response and improves disease resistance (Gatlin, 2015). Administration of prebiotics has been reported to have a positive effect on beneficial gut bacteria such as lactic acid bacteria (LAB) which are involved in gut metabolism and health benefits of the host (Carbone & Faggio, 2016).

Prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Ringo, Olsen, Gifstad, Dalmo, & Amhund, 2010). Compounds which have been shown to have prebiotic characteristics include fructooligosaccharides (FOS), mannanoligosaccharides (MOS), galactooligosaccharides (GOS), xylooligosaccharides (XOS), isomaltooligosaccharides, (IMO) and inulin (Ringo et al., 2010). Although these are mostly plant derived additives and are often not naturally present in fish diets, especially in carnivorous fish, the prebiotic potential of oligosaccharides and other dietary fibres may have interesting applications in aquaculture to stimulate gut health, and the presence of beneficial gut bacteria will aid in suppressing the potentially deleterious bacteria (Ringo et al., 2010). Inulin is one of the most commonly used prebiotics in animal feeds it is produced by different plants sources and appears to have a capacity to alter fish gut microbiota (Roberfroid, 2005). Dietary supplementation of inulin has been shown to enhance growth and improving health in fish (Blanca, Luna-Gonzalez, Jesus, Del Carmen, & Hector, 2013; Burr, Hume, Ricke, Nisbet, & Gatlin, 2010). The utility of dietary prebiotic inulin in the diet of seabass has not been explored so far. Consequently, the present study investigated the effect of supplementation of inulin in the diet of Asian seabass juveniles.

2 | MATERIALS AND METHODS

2.1 | Diet preparation

The experimental diets were formulated based on the nutritional requirement of Asian seabass using commercially available feed ingredients. The dietary inulin was supplemented at the levels of 5, 10, 15, and 20 g/kg. Inulin unsupplemented 0 (control) diet was also prepared to contain 400 g/kg protein and 90 g/kg lipid. Feed ingredients were ground into a fine powder through a 0.5-mm mesh sieve. The diet mix was made into a soft dough by adding the required quantity of water. The dough was then steam cooked and pelletized in a pelletizer (Unique CNC Engineers, Chennai, India) using a 1.0-mm die. The experimental diets were prepared in the feed mill of ICAR-Central Institute of Brackishwater Aquaculture (CIBA). After drying, the experimental diets were crumbled and sieved into the appropriate pellet size, packed in airtight containers and were stored at −20°C until use. The ingredients and composition of the basal diet are given in (Table 1).

2.2 | Prebiotic

The inulin was provided by (Adept impexpvt Ltd, Agra, Uttar Pradesh, India). Prebiotic inulin is a powdered food ingredient that contains mainly fructans. It is a standard form of chicory inulin. The degree of polymerization (DP) of standard inulin ranges from 2 to 60. The other components are mainly glucose, fructose, and sucrose.

TABLE 1 Ingredients and proximate composition of experimental diets g/kg

Diets	Control (0 g)	5 g/kg	10 g/kg	15 g/kg	20 g/kg
Ingredients g/kg					
Fish meal[a]	400	400	400	400	400
Soya meal	250	250	250	250	250
Wheat	130	130	130	130	130
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 Mineral mixture[b]	30	30	30	30	30
Binder[c]	10	10	10	10	10
Cellulose	20	15	10	5	0
Inulin[d]	0	5	10	15	20
Proximate composition					
Moisture	78.6	78.3	78.0	78.3	75.1
Crude protein	401.2	404.3	403.5	402.1	402.4
Crude lipid	88.1	88.3	88.6	88.7	88.6
Crude fibre	23	26.3	26.4	26.8	26
Total ash	131.1	143.8	144.5	145.1	145.9
Nitrogen free extract	278	259	259	259	262

Notes: [a]Sardine fishmeal and fish oil. Bismi fisheries, Mayiladuthurai, Tamil Nadu, India.
[b]Commercially sourced premix and each kg contains Vitamin A—2,000,000 IU, Vitamin D—40,000 IU, Vitamin E—300 U, Vitamin K—450 mg, Riboflavin—800 mg, Panthothenic acid—1 g, Nicotinamide—4 g, Vitamin 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 Agri nutrition Asia Pvt Ltd, Singapore.
[d]Inulin—(Adept Impex Pvt Ltd, Agra, Uttar Pradesh, India).

2.3 | Feeding trial

Healthy Asian seabass fingerlings were procured from a Muttukkadu fish hatchery, Chennai; fingerlings were fed with the control diet for 1 week before to the feeding trial to make the animals acclimate the experimental diet and environmental conditions. The fingerlings initial average body weight: 7.14 ± 0.05 g were randomly distributed into fifteen 1000-L oval fibreglass reinforced plastics (FRP) tanks at the rate of 20 fish per tank, with three replications for each treatment. The feeding trial was conducted for 60 days in a flow-through system at the Muttukadu Experimental Station of (CIBA), Chennai, India. Fish were offered experimental feed at the rate of 2.5%–3.0% of total biomass, twice a day (10:00 and 16:00 hours). The stipulated quantity of feed per tank was offered by standing over a period and feeding the fish till it attains satiation and feed intake was precisely monitored to ensure that there are no variations in feed consumption among the treatments. At the end of the feeding period, the uneaten feed particles were collected from the tank after an hour of feeding and were dried overnight at 60°C in a hot air oven to compute the feed intake on a daily basis. This procedure would ensure that all the fish would take the required quantity of feed. Animals were weighed individually at the start and end of the experiment while bulk weighing was carried out at fortnightly intervals following a 24-hr starvation period to ascertain the increase in weight. The fish that died during the experimental period have been collected and weighed and taken for calculation of growth metrics. Fish were maintained under a natural photoperiodicity (12 hr L:12 hr D).

2.4 | Water quality parameters

The water quality parameters viz., salinity (28–349 g/L), temperature (26–28°C), dissolved oxygen (6.0–7.3 mg/L), pH (7.2–8.1), and total ammonia nitrogen (0.08–0.11 mg/L) was recorded using conventional methods (APHA/AWWA/WPCF, 1998). Ultraviolet treated water was used for the experiment.

2.5 | Chemical analysis

The proximate composition of the ingredients and experimental diets was analysed by following standard procedures (AOAC, 2012). Moisture content was estimated by gravimetric analysis after oven drying at 105°C for 12 hr. 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 hr in a muffle furnace. Crude fibre was estimated gravimetrically after acid and alkali digestion and loss in mass by combustion at 600°C for 3 hr. Nitrogen free extract (NFE) was calculated from 1,000 − (crude protein + crude lipid + crude fibre + total ash). All the chemical analyses were done in triplicate and reported on a dry matter basis.

2.6 | Blood sampling

At the end of the experiment, fish were starved for 24 hr before blood sampling. Three fish from each replicate were randomly chosen and anaesthetized in diluted MS-222 (tricaine methanesulphonate) at the concentration of 150 mg/L. Blood samples were taken from the caudal vein using heparinized 2 ml syringe with a 26-G needle. One part of the blood sample was transferred into tubes containing heparin as an anticoagulant for the determination of haematological and immunological assay. The remaining blood was separated by centrifugation, and the supernatant was pooled and stored at −70°C for further analysis (Jalali, Ahmadifar, Sudagar, & Azari Takami, 2009).

2.7 | Haematological parameters

The haematological parameters red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb) mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were analysed according to Blaxhall and Daisley (1973). Packed cell volume (PCV) was measured using the standard microhematocrit method and reported as percentages described by England and Walford (1972).

2.8 | Biochemical parameters

The biochemical parameters such as serum glucose, urea, cholesterol, triglycerides, and total protein were estimated using the respective commercial kits obtained from Sigma-Aldrich in a UV-spectrophotometer (Shimadzu, UV-1800) at 595, 340, 570, and 540 nm, respectively, according to the accredited methodologies given by Sigma-Aldrich. Activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel (1957). Alkaline phosphatase (ALP) was determined using an enzymatic method described by Borges, Scotti, Siqueira, Jurinitz and Wassermann (2004). Lactate dehydrogenase (LDH) was assayed by the method of Anon (1984).

2.9 | Immunological parameters

Serum lysozyme activity was determined by turbidimetric assay according to the method described by Ellis (1990). Alternative complement pathway (ACP) was performed as described by Sunyer and Tort (1995). Superoxide dismutase activity (SOD) assay was determined according to the method described by Misra and Fridovich (1972). The Nitroblue tetrazolium (NBT) assay was carried out following the protocol of Anderson and Siwicki (1995).

2.10 | Intestinal and liver histology

At the end of feeding experiment, three fish per treatment group were taken for histological studies and fixed in phosphate–buffered formalin. The tissues samples were processed as per the protocols described by Roberts (2001) and sectioned using microtome Leica

RM 2245. Photomicrographs of intestines and liver sections were recorded using an Olympus CX41 microscope with digital camera C7070 attachment.

2.11 | Statistical analysis

All treatments were replicated three times, and the experimental unit was a tank with 20 fish. All data were subjected to normality and homogeneity of variances. After assessing this comparison of means was carried out using ANOVA. Data were analysed using one-way ANOVA followed by Duncan's multiple range test (post hoc) to compare the means of the treatments. Differences were considered significant at ($p < 0.05$). SPSS ver. 16.0 was used for data analysis.

3 | RESULTS

3.1 | Haematological parameters

Haematological parameters of seabass fingerlings fed with different levels of dietary inulin are shown in Table 2. The results showed that red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) showed significant differences ($p < 0.05$) between control and inulin supplemented diets. The fish fed with 5–20 g/kg inulin supplemented diets showed significantly higher values than the control diet and inferred that inulin supplementation had influenced the haematological parameters.

3.2 | Biochemical parameters

The effect of dietary inulin supplementation on seabass fingerlings blood profiles is displayed in Table 3. The analysis of serum samples glucose, urea, cholesterol, triglyceride, AST, ALT, ALP, and LDH of seabass fed with inulin supplemented diets showed significant differences ($p < 0.05$) between control and various treatments. No significant difference ($p > 0.05$) was observed for total protein among different treatments. However, glucose, triglyceride, AST, and ALP showed significantly higher values in inulin supplemented diets.

3.3 | Immunological parameters

The effects of varying levels of dietary inulin supplementation on the innate immune responses of seabass juvenile are shown in (Figures 1, 2, 3, 4). The lysozyme activity was significantly ($p < 0.05$) affected by dietary 20 g/kg inulin supplementation. Significantly ($p < 0.05$) highest ACP, SOD, and NBT activity was observed in fish fed with 15–20 g/kg inulin supplemented diet compared to rest of the treatments. However, there was no significant difference ($p > 0.05$) observed in lysozyme and NBT activity between the control, 5 and 10 g/kg inulin supplemented groups.

3.4 | Histological observations

Histological observations on the post fed experimental animals revealed that the intestine of fish fed with control diet appeared normal and most of the villi appeared straight without any bifurcations. The secretory cells were found to be distributed normally throughout the villi. However, inulin supplementation showed numerous side branches of villi. These villi were found to be occupying the whole lumen. In the fish fed with 20 g/kg inulin, each villus had additional side branches indicating the hyperplasia in the villus epithelium which was seen on both the sides of the villus indicating the increased absorptive surface for nutrients. The mucosal layer of the villus had a normal distribution of the secretory or goblet cells (Figure 5). Similarly, the hepatic tissue appeared normal in a regular-shaped morphology of the hepatocytes around sinusoidal spaces and supplementation of inulin showed vacuolations in few hepatocytes with centrally placed nuclei, resembling the accumulation of glycogen deposits (Figure 6).The hepatic tissues showed severe changes with hepatocytes, the presence of cytoplasmic vacuoles displacing the nuclei to the periphery of the cell indicating accumulation of lipids.

4 | DISCUSSION

This is the first study to investigate the effect of inulin on histology, immuno-haematological, and biochemical parameters of Asian seabass (*Lates calcarifer*) fingerlings. The haematological parameters

TABLE 2 Haematological parameters of seabass fed experimental diets supplemented with varying levels of inulin for 60 days

Parameters	Control (0 g)	5 g/kg	10 g/kg	15 g/kg	20 g/kg
RBC (10^6 mm^{-3})	$3.46^{a}\pm0.03$	$3.65^{b}\pm0.04$	$3.73^{c}\pm0.04$	$3.65^{b}\pm0.04$	$3.66^{b}\pm0.03$
WBC (10^3 mm^{-3})	$7.8^{a}\pm0.10$	$8.2^{b}\pm0.12$	$8.33^{b}\pm0.15$	$8.3^{b}\pm0.21$	$8.3^{b}\pm0.10$
Hb (g/dl)	$7.2^{a}\pm0.10$	$7.4^{b}\pm0.06$	$7.2^{a}\pm0.10$	$7.8^{c}\pm0.06$	$7.5^{b}\pm0.10$
PCV (%)	$34.66^{a}\pm0.15$	$38.66^{c}\pm0.32$	$36.30^{b}\pm0.10$	$36.00^{b}\pm0.00$	$36.20^{b}\pm0.10$
MCV(fl)	$101.53^{a}\pm0.35$	$102.36^{b}\pm0.25$	$102.23^{b}\pm0.15$	$103.46^{c}\pm0.21$	$104.63^{d}\pm0.15$
MCH (pg)	$19.46^{a}\pm0.29$	$20.33^{b}\pm0.15$	$20.56^{bc}\pm0.29$	$20.46^{bc}\pm0.21$	$20.86^{c}\pm0.06$
MCHC (g/dl)	$18.40^{a}\pm0.26$	$19.36^{b}\pm0.26$	$19.13^{b}\pm0.06$	$20.46^{c}\pm0.40$	$21.53^{d}\pm0.38$

Notes: All values are mean ± *SE* of three observations.
Mean bearing different superscript in a row differ significantly ($p < 0.05$).
RBC: Red blood cells; WBC: White blood cells; Hb: Haemoglobin; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration.

TABLE 3 Biochemical parameters of seabass fed experimental diets supplemented with varying levels of inulin for 60 days

Parameters	Control (0 g)	5 g/kg	10 g/kg	15 g/kg	20 g/kg
Glucose (mg/dl)	$47.30^{a}\pm5.63$	$62.96^{c}\pm3.55$	$50.86^{b}\pm2.65$	$70.60^{d}\pm4.07$	$84.73^{e}\pm4.18$
Urea (mg/dl)	$19.53^{ab}\pm3.51$	$13.03^{a}\pm2.81$	$31.33^{c}\pm5.18$	$21.46^{b}\pm5.30$	$14.03^{ab}\pm2.64$
Cholesterol (mg/dl)	$139.16^{a}\pm4.11$	$163.60^{b}\pm4.40$	$158.66^{b}\pm5.40$	$157.16^{b}\pm6.16$	$170.43^{c}\pm4.84$
Triglyceride (mg/dl)	$247.96^{a}\pm4.64$	$262.73^{b}\pm5.78$	$260.10^{b}\pm6.40$	$286.20^{c}\pm7.05$	$278.13^{c}\pm5.58$
Total protein (g/dl)*	3.88 ± 0.83	3.59 ± 0.64	2.76 ± 0.82	2.94 ± 0.82	3.71 ± 0.99
AST (U/L)	$192.9^{a}\pm4.45$	$247.7^{c}\pm3.12$	$197.2^{a}\pm2.80$	$239.63^{b}\pm5.43$	$264.73^{d}\pm3.78$
ALT (U/L)	$47.83^{b}\pm1.83$	$32.66^{a}\pm6.81$	$36.30^{a}\pm2.86$	$39.10^{ab\pm}4.40$	$38.06^{a}\pm6.31$
ALP (U/L)	$17.53^{a}\pm4.65$	$17.7^{a}\pm3.29$	$27.36^{b}\pm1.32$	$25.80^{b}\pm2.52$	$29.43^{b}\pm3.78$
LDH (UL)	$1079.43^{ab}\pm10.10$	$1062.63^{a}\pm11.49$	$1095.76^{b}\pm5.60$	$1186.83^{c}\pm9.19$	$1185.33^{c}\pm10.26$

Notes: All values are mean ± *SE* of three observations.
Mean bearing different superscript in a row differ significantly ($p < 0.05$).
AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase.
*Nonsignificant $p > 0.05$.

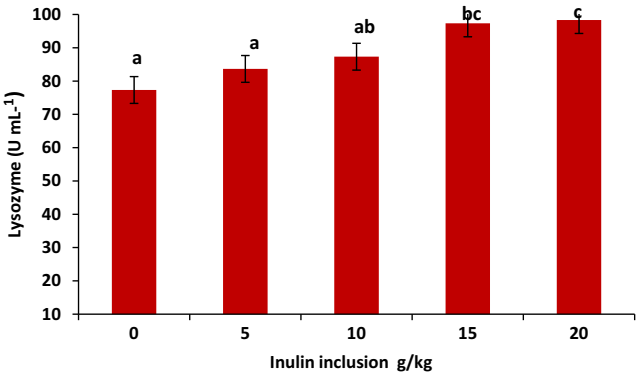

FIGURE 1 Lysozyme activity of seabass fed experimental diets supplemented with varying levels of inulin for 60 days (mean ± *SD*). Bars assigned with different superscripts are significantly different ($p < 0.05$) [Colour figure can be viewed at wileyonlinelibrary.com]

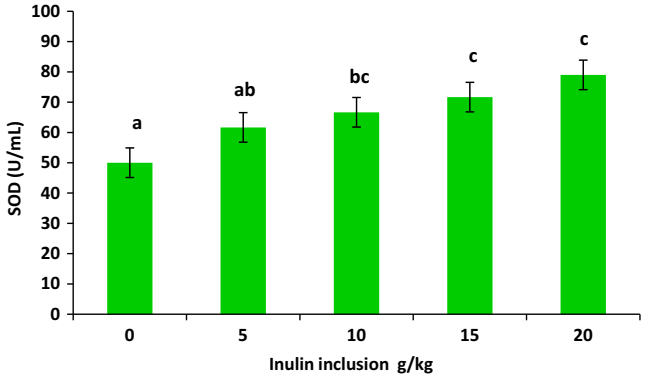

FIGURE 3 SOD activity of seabass fed experimental diets supplemented with varying levels of inulin for 60 days (mean ± *SD*). Bars assigned with different superscripts are significantly different ($p < 0.05$) [Colour figure can be viewed at wileyonlinelibrary.com]

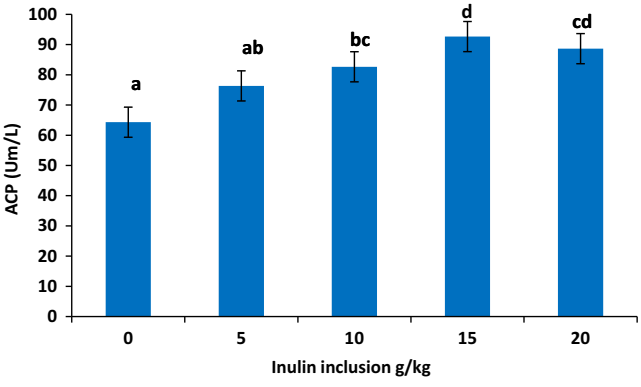

FIGURE 2 ACP activity of seabass fed experimental diets supplemented with varying levels of inulin for 60 days (mean ± *SD*). Bars assigned with different superscripts are significantly different ($p < 0.05$) [Colour figure can be viewed at wileyonlinelibrary.com]

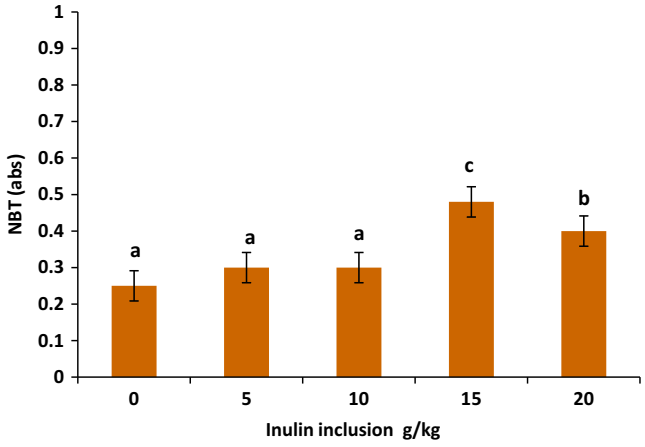

FIGURE 4 NBT assay of seabass fed experimental diets supplemented with varying levels of inulin for 60 days (mean ± *SD*). Bars assigned with different superscripts are significantly different ($p < 0.05$) [Colour figure can be viewed at wileyonlinelibrary.com]

showed that RBC, WBC, Hb, PCV, MCV, MCH, and MCHC levels increased significantly in the fish fed with inulin supplemented diets. Similar to our results Ahmadifar, Akrami, Ghelichi and Mohammadi (2010) reported that the supplementation of diets with 1% inulin significantly increased WBC and lymphocyte values in beluga juvenile. With increasing levels of supplementation of inulin, the mean values of MCH and MCHC increased but the mean value of WBC,

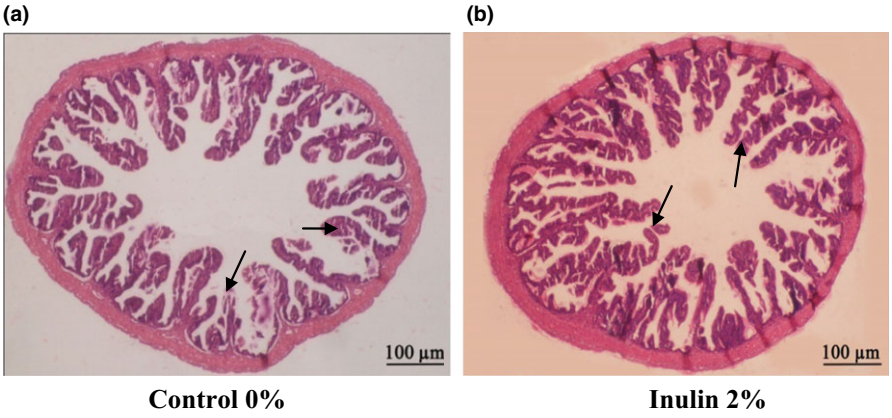

FIGURE 5 Comparative light photomicrograph sections from the anterior intestinal region presenting the positive effect of inulin supplementation. Section (a) Fish fed the control diet (0% inulin). Arrows indicate uneven intestinal folding. Section (b) Fish fed with inulin supplemented diet (20 g/kg). Arrows indicate improved intestinal folding. H&E stain [Colour figure can be viewed at wileyonlinelibrary.com]

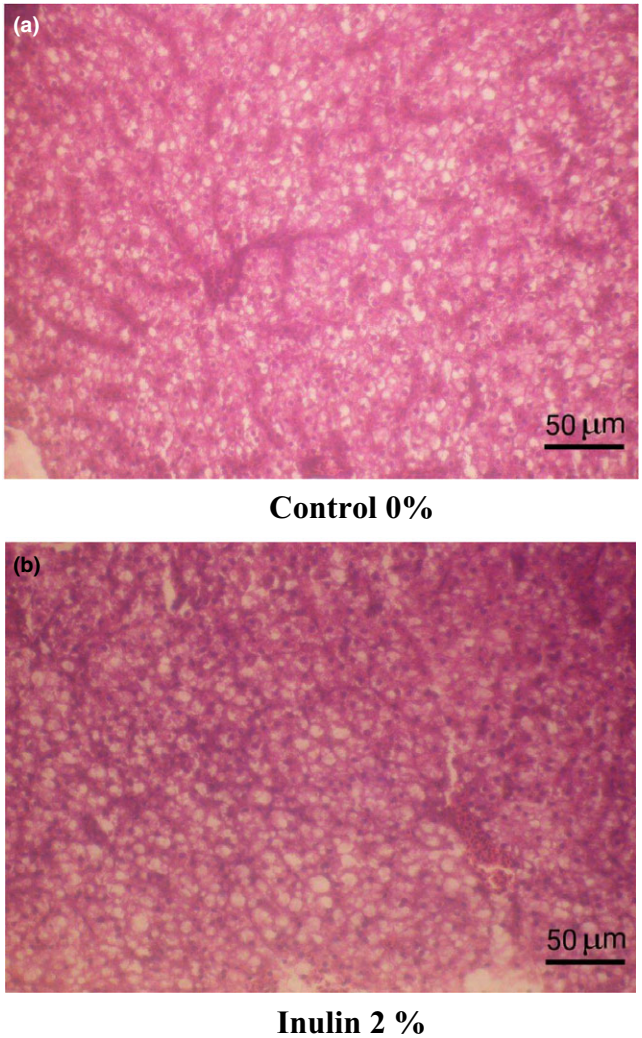

FIGURE 6 Comparative light photomicrograph of seabass liver sections. Section (a) Fish fed with control diet with normal hepatocytes. Section (b) Fish fed with inulin supplemented diet (20 g/kg) showing vacuolations in hepatocytes and glycogen deposition. H&E stain [Colour figure can be viewed at wileyonlinelibrary.com]

RBC, Hb, PCV, and MCV decreased. On the contrary, Akrami, Abdol Majid, Abbas and Abdol Mohammad (2009) reported a nonsignificant difference in the levels of RBC and MCH between treatments in juvenile beluga fed with inulin in the 8-week feeding trial. The highest value of WBC was observed in fish fed with the diet containing 1% prebiotic inulin (Akrami et al., 2009). Hoseinifar, Mirvaghefi, MojaziAmiri, Rostami and Merrifield (2011) reported a non-significant improvement in haematological parameters (WBC, haemoglobin and lymphocyte) in beluga (*H. huso*) fed on dietary prebiotics fructooligosaccharide. Previous studies have been reported in Channel catfish (*Ictalarus punctatus*; Welker, Lim, Yildirim-Aksoy, Shelby, & Klesius, 2007), Nile tilapia (*Oreochromis niloticus*; Sado, Bicudo, & Cyrno, 2008), Grass carp (*Ctenopharyngodonidella*; Shaker Khoshroudi, 2011), and Asian seabass (*Lates calcarifer*; Syed Raffic Ali, Ambasankar, Ezhil Praveena, Nandakumar, & Syamadayal, 2015) haematological parameters were not significantly affected by the dietary mannan oligosaccharide. The analysis of haematological parameters is a valuable guide in assessing the condition of aquatic organisms (Akrami et al., 2013). Leucocytes are important cells that can stimulate immune responses in fish. These cells produce antibodies and can perform macrophages activities (Jalali et al., 2009). The determination of blood parameters RBC count is used for assessing fish health. (Bhaskar & Rao, 1985). Haemoglobin serves to transport oxygen from gills to different tissues of the fish in the form of oxyhaemoglobin and carbon dioxide from tissue to the gills in the form of carboxyhaemoglobin. Blaxhall and Daisely (1973) have reported the possibility of using PCV as a tool in aquaculture and fishery management for checking anaemic condition. The haematological parameters of fish are reported to be affected by a range of factors, which include species, size, age, physiological status, environmental conditions, and dietary regime (e.g., quality and quantity of food, dietary ingredients, protein sources, vitamins, and prebiotics) Hoseinifar et al. (2011).

The present study revealed that using more than 10 g/kg inulin had adverse effects on some biochemical parameters of seabass fingerlings. On the contrary Akrami et al. (2009) in Juvenile Beluga, (*Huso huso*) reported that cholesterol, glucose, triglycerides, uric acid, AST, ALT, ALP, and LDH activities were not significantly affected by inulin supplementation at 1%, 2%, and 3% diets in 8 weeks feeding trial. However, plasma total protein content of fish fed with diets containing prebiotic inulin was lower than that of the basal group. Refstie et al. (2006) reported that the glucose, cholesterol and triglyceride levels were not affected by the dietary inulin

supplementation in Atlantic salmon. Hoseinifar et al. (2011) reported that serum lactate dehydrogenase (LDH), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities did not significantly differ between treatments on the blood profiles of beluga (*Huso huso*) juveniles fed with dietary oligofructose (1%, 2%, and 3%). In the present study, the amount of glucose ranged from 47 to 84 mg/dl and the highest level was found in the fish fed with inulin 20 g/kg. In this scenario, fish performs the biochemical reaction of gluconeogenesis and changes tissues glycogen to glucose and deposits it into the blood. Protein is the most important compounds in serum and its concentration is considered as a basic index for fish health status (Alexander, Sahu, Pal, & Akhtar, 2011). Increases in serum protein contents are usually thought to be associated with a stronger innate immune response (Magnadottir, 2006). The serum activity of ALT and AST varies depending on fish species (Akrami et al., 2013). The increase in plasma AST and ALT may be connected to a stress condition. It appears that fluctuations in biochemical variables may be species-specific and depend on many factors viz., inclusion rates of prebiotics in diet, type of ingredients, and the rearing period (Anon, 1984; Ta'ati, Soltani, Bahmani, & Zamini, 2011).

In the present study, 15–20 g/kg inulin supplementation significantly enhanced the immunological parameters. Similar to our results (Ibrahem, Fathi, Mesalhy, & Abd El-Aty, 2010) reported a significant increase in lysozyme and NBT activity in inulin supplemented group, in gilthead sea bream at 2 months experiment. Similarly, Cerezuela, Cuesta, Meseguer and Angeles (2008) reported a significant increase in ACP activity in inulin supplemented group, in a 2 months experiment in gilthead sea bream. Significantly higher SOD activity was reported at 10 g/kg prebiotic fructooligosaccharide supplementation in seabass juveniles for 45 days (Syed Raffic Ali, Ambasankar, Ezhil Praveena, Nandakumar, & Syamadayal, 2016). On the contrary, Zhang et al. (2013) reported that plasma ACP, SOD and lysozyme activities were not affected by supplementation of FOS in Triangular bream (*Megalobrama terminalis*). Lysozyme is an imperative defense molecule of the innate immune system which plays an important role in mediating protection against microbial intrusion (Li et al., 2012). The high ACP activity might be attributed to the enhanced liver function, which was supported by the fact that liver is the main source of complement proteins and hepatic enhancement might, therefore, affect positively on complement (Holland & Lambris, 2002). SOD is the important biochemical parameters for antioxidant defense (Kohen & Nyska, 2002). The measurement of this activity can indicate the antioxidant status of fish, as can also serve as biomarkers of oxidative stress (Hadas & Stankiewicz, 1998). SOD is a cytosolic enzyme that is specific for scavenging superoxide radicals and is involved in protective mechanisms in tissue injury following oxidative process and phagocytosis (Olsen, Myklebust, Kryvi, Mayhew, & Ringo, 2001).

Histological analysis revealed that inulin supplementation resulted in increased microvilli density in the anterior and posterior intestinal regions. The analysis suggests that inulin supplementation can improve microvilli structures. The significant increase in microvilli in the anterior and posterior intestine observed in the study signifies the beneficial effect of inulin supplementation. These changes in the intestinal villi would help to improve nutrient utilization there by resulting in better growth performance. Glycogen deposition in the liver tissue revealed that inulin supplementation significantly increased the quantity of glycogen in the hepatocytes. Glycogen stored in hepatocytes can easily be used as energy sources in case of interrupted feeding which may arise in commercial rearing facilities especially during stress conditions. Similar to our results Tiengtam, Khempaka, Paengkoum and Boonanuntanasarn (2015) reported that dietary supplementation with inulin (5.0 g/kg) and Jerusalem artichoke (5.0 and 10.0 g/kg) resulted in greater villus height in all parts of the intestine, although a significant increase in villus height was observed only in the anterior and middle parts. However, the effect of dietary inulin on carnivorous fish appears to be different. Contrary with this work Olsen et al. (2001) fed with high level 15% dietary inulin to on-growing Artic charr (*Salvelinus alpinus*) after 4 weeks of feeding, histological analysis revealed that inulin caused destructive effects in the lay-out and structure of microvilli in the distal intestine. Refstie et al. (2006) reported that histology of the distal intestine revealed that inulin does not induce any morphological changes in Atlantic salmon. Cerezuela et al. (2013) reported that inulin has a negative impact on the intestinal morphology of gilthead sea bream, as evident by different signs of oedema and inflammation. Fish fed the control diet had a normal gross morphology of the intestinal wall, so the observed alterations must be attributed only to the addition of prebiotics. Caspary (1992) reported that the intestinal morphometric study revealed no significant effect of inulin on the intestinal absorptive area. It has been proposed that the increase in the length of the villi implied an increase in surface area which facilitates the better absorption of available nutrients. Similar to our results the beneficial effect of prebiotics MOS supplementation has been reported by (Anguiano, Pohlenz, Buentello, & Gatlin, 2013; Dimitroglou et al., 2010; Torrecillas et al., 2007, 2011).

Results from this study showed that inulin supplementation alters the morphological characters of the intestine and has got the beneficial effect on immuno-haematological and biochemical parameters of seabass. Furthermore, it can be concluded that inclusion of inulin in the range of 15–20 g/kg in the diet of seabass would be beneficial. However, further studies are required to conclusively ascertain the prebiotic effect of inulin supplementation and to arrive at the optimal level of inclusion for enhancing the immune responses and disease resistance.

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