Aquaculture Research

Aquaculture Research, 2015, 1–10 doi: 10.1111/are.12933

Effect of dietary mannan oligosaccharide on growth, body composition, haematology and biochemical parameters of Asian seabass (*Lates calcarifer*)

Syed Raffic Ali, Kondusamy Ambasankar, Ezhil Praveena, Sambasivam Nandakumar & Jagabatula Syamadayal

Central Institute of Brackishwater Aquaculture (ICAR), Chennai, India

Correspondence: K Ambasankar, Central Institute of Brackishwater Aquaculture, 75 SanthomeHigh Road, R.A. Puram, Chennai 600028, India. E-mail: ambasankar@ciba.res.in

Abstract

A feeding trial was conducted to study the effect of mannan oligosaccharide (MOS) supplementation on growth, body composition, haematology, biochemical parameters and histology of Asian seabass (*Lates calcarifer*) fingerlings (8.13 ± 0.06 g average weight). Mannan oligosaccharide was supplemented at five different concentrations viz., 0%, 0.5%, 1%, 1.5% and 2% levels in the diet (40% protein and 9% lipid) of *L. calcarifer*. The results of the 60 days feeding trial showed that there was a significant ($P < 0.05$) increase in the final body weight, weight gain (WG), WG%, average daily gain, survival, specific growth rate, daily growth coefficient, hepatosomatic index and viscerosomatic index in the fish fed diet containing 1% MOS. Whole body composition of post-fed animals showed non-significant differences ($P > 0.05$) among the various treatment groups. The analysis of haematological parameters showed that there was no significant ($P > 0.05$) differences among different treatments but for the haemoglobin content which was significantly ($P < 0.05$) higher in the fish fed 1% MOS. Biochemical parameters revealed that glucose, urea, cholesterol and triglyceride content showed significant ($P < 0.05$) difference between control and MOS-supplemented group. Histological observations of post-fed animals revealed that MOS supplementation resulted in increased absorptive surface area of the intestine and increased glycogen deposition in liver. The result of this experiment infers that MOS supplementation has got a beneficial effect in the diet of seabass and supplementation at 1% level is optimal for improving the growth.

Keywords: Asian seabass, growth, histology, microbiota, microvilli

Introduction

Asian seabass (*Lates calcarifer*) commonly known as bhetki or barramundi, is an economically important candidate species and it is being widely cultured in Southeast Asia and Australia under extensive or intensive system in fresh, brackish and marine water resource (Glencross 2006). In India, it is being considered as a potential alternate candidate species for coastal aquaculture (Nandakumar, Ambasankar, Syamadayal, Raman & Ali 2013). Diseases are one of the primary limiting factors for large scale propagation of aquaculture. As an alternative strategy to antibiotics, prebiotics have recently attracted extensive attention in aquaculture (Ringo, Olsen, Gifstad, Dalmo & Amhund 2010). Prebiotics are non-digestible 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 (Manning & Gibson 2004). Manipulation of microbial population in the intestinal tract of aquatic animals through the use of prebiotics is the novel approach to improve the health and growth of the animal (Ringo *et al.* 2010). Mannan oligosaccharides (MOS) are structurally complex carbohydrates derived from yeast cell walls. These materials contain mannose as the primary carbohydrate element (Ta'ati, Soltani, Bahmani & Zamini 2011). Functional dietary supplements are developed in order to protect fish health and improve performance. The intestinal microbiota

plays an important role in the nutrition and health of the host organism. The intestine is an internal organ with high bacterial load and is a potential port of entry for pathogens. Potential pathogenic bacteria are part of the intestinal microbiota of every healthy organism (Gatesoupe 1999) and if the conditions within the intestine become favourable (i.e. the host becomes stressed or is subjected to poor nutrition etc.) then there is potential for pathogenic proliferation, translocation and ultimately infection of the host organism. Hence, an attempt was made to study the effect of supplementation of MOS in the diet of Asian seabass (*L. calcarifer*) on growth, body composition, haematology, biochemical parameters and histology.

Materials and methods

Preparation of experimental diets

The effect of supplementation of MOS was studied by supplementing it in the standard seabass diet (CIBA BHETKIAHAR) at five different concentrations viz., 0%, 0.5%, 1%, 1.5% and 2% levels. The ingredient and proximate composition of the experimental diets are given in the (Table 1). Dry solid feed ingredients were powdered in an electrical grinder and passed through a 0.5-mm sieve. They were mixed together along with additives and homogenized thoroughly in an electrical blender. The diet mix was made into soft dough by adding required quantity water at about 40%. The dough was steam cooked (at atmospheric pressure) for 5 min, cooled and pelletized in a hand pelletizer using a 2.0-mm die. The diet was dried in a hot air oven at 70°C to moisture content of less than 9% and stored in a dessicator until use. The proximate composition of the ingredients, diets and experimental animals were analysed by following standard procedures of AOAC (2012).

Fish rearing and experimental design

Hatchery-bred and farm-reared Asian seabass fingerlings were procured from the farm located at Kulathumedu village near Pulicat Lake in Tamil Nadu. They were transported to the nutrition wet-laboratory and were acclimatized to the laboratory conditions for 2 weeks during which they were fed CIBA- Bhetkiahar (a pelleted feed developed for seabass at CIBA). The fingerlings (average body weight: 8.13 ± 0.06 g) were randomly distributed into fifteen oval 1000-L fibre-reinforced plastic tanks. The experiment was carried out in a completely randomized design with three replicates for each treatment constituting 15 animals per replicate. The tanks were supplied with sand-filtered seawater with provisions for continuous aeration through air diffuser stones. Throughout the trial, water in the tanks (about 80%) was exchanged twice a day, in the morning and evening. Fish were hand fed *ad libitum* twice daily (10.00 and 16.00 hours) and after 30 min, 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 while bulk weighing was carried out at fortnightly intervals to ascertain the increase in weight. Fish were maintained under a natural photoperiodicity (12 h L: 12 h D). Water quality parameters viz.

Table 1 Ingredient and proximate composition (%) of experimental diets containing varying levels of MOS

Diets	MOS 0%	MOS 0.5%	MOS 1%	MOS 1.5%	MOS 2%
Ingredients (%)					
Fish meal*	40	40	40	40	40
Soybean meal	25	25	25	25	25
Wheat	14	14	14	14	14
Rice	5	5	5	5	5
Maize	5	5	5	5	5
Fish oil*	4	4	4	4	4
Lecithin	1	1	1	1	1
Vitamin & minerals†	3	3	3	3	3
Binder‡	1	1	1	1	1
Cellulose	2	1.5	1	0.5	0
MOS§	0	0.5	1	1.5	2
Proximate composition					
Moisture	8.75	8.26	8.44	8.20	8.24
Crude protein	40.32	40.37	40.36	40.32	40.46
Crude lipid	8.83	8.82	8.85	8.81	8.84
Crude fibre	2.16	2.12	2.27	2.12	2.15
Total ash	14.08	14.01	14.47	14.12	13.10
NFE	25.86	26.42	25.61	26.43	27.21

*Sardine fishmeal and fish oil. Bismi fisheries, Mayiladuthurai, Tamil Nadu, India.
†Commercially sourced premix and each kg contains Vitamin A – 2 000 000 IU, Vitamin D – 400 000 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, Vadodara, Gujarat, India.
‡Pegabind, Bentoli Agri nutrition Asia, Singapore.
§MOS Alltech,-Bangalore.

temperature, salinity, pH, dissolved oxygen and total ammonia nitrogen were analysed on a weekly basis and the values ranged from 26 to 29°C, 28–31 ppt, 7.4–8.2, 6.0–7.3 mg L^{-1} and 0.08–0.11 mg L^{-1} respectively.

Blood sample collection

At the end of the experiment, three fish were randomly chosen from each tank and were anaesthetized using clove oil. 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-heparanized tubes to analyse haematological and biochemical assay respectively. For serum separation blood samples in the non-heparanized tubes were centrifuged at 1000 *g* for 5 min to separate the serum. Serum samples were preserved at -20°C until use.

Haematological parameters

The heparinized blood samples were used for analysis of haematological parameters. Red blood cells (RBC) and white blood cells (WBC) counts were determined using a Neubaeur haemocytometer according to (Feldman, Zinkl & Jian 2000). Packed cell volume (PCV) was measured using the standard microhematocrit method and reported as percentages described by (England, Walford & Waters 1972). Haemoglobin (Hb) levels were estimated by cyanomethaemoglobin method (Blaxhall & Daisley 1973).The erythrocytes indices mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to Wintrobe (1974).

Biochemical parameters

Serum glucose was estimated using a commercial kit (Sigma Diagnostics, Baroda, India) by the method of Trinder (1969). Urea in the serum was estimated by using the diagnostic kit based on the method of Fawcett and Scott (1960). Serum cholesterol levels were estimated according to method of Parekh and Jung (1970). Triglyceride levels were estimated according to the method of Rice (1970). Total protein was estimated according to the method of Lowry, Rosebrough, Farr and Randall (1951). Serum albumin was estimated by bromocresol green (BCG) dye-binding method using a kit (Siemens, Vadodara, India). Globulin was calculated by subtracting albumin value from total plasma protein. Activities of (AST) aspartate aminotransferase (ALT) alanine aminotransferase 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).

Intestinal and liver histology

At the end of feeding experiment two 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 (Leica Biosystems, Nussloch, GmbH, Germany). Photomicrographs of intestines and liver sections were recorded using an Olympus CX41 microscope with digital camera C7070 attachment (Tokyo, Japan).

Statistical analysis

Data were analysed using one-way ANOVA to compare significant differences between treatments, where as Duncan's multiple range tests was used to compare the means of the treatments. All the data were analysed using SPSS version 16.0 software (SPSS, Chicago, IL, USA).

Results

Growth performance and survival

The growth performance and survival of seabass fed MOS supplemented diets is presented in (Table 2). The results showed that there was a significant ($P < 0.05$) increase in the final body weight, weight gain (WG), WG%, average daily gain (ADG), specific growth rate (SGR) and daily growth coefficient (DGC) of fish fed 1% MOS-supplemented diet compared to the rest of the treatments. Significantly ($P < 0.05$) better FCR was recorded in 1% MOS-supplemented diet. Survival was significantly ($P < 0.05$) different between control and MOS-supplemented groups. Group fed 1% MOS-supplemented diet showed a significantly higher survival (93.33%). However, this was

Table 2 Growth performance and survival of seabass fed experimental diets supplemented with varying levels of MOS for 60 days

Parameters	MOS 0%	MOS 0.5%	MOS 1%	MOS 1.5%	MOS 2%
IBW*	8.13 ± 0.06	8.08 ± 0.07	8.17 ± 0.04	8.15 ± 0.06	8.03 ± 0.07
FBW	40.53^{a} ± 0.50	42.30^{b} ± 0.40	43.86^{c} ± 0.75	43.56^{c} ± 0.61	43.66^{c} ± 1.08
WG	32.39^{a} ± 0.46	34.21^{b} ± 0.41	35.69^{c} ± 0.77	35.41^{c} ± 0.55	35.39^{c} ± 1.12
WG%	398.14^{a} ± 3.75	423.10^{b} ± 6.79	436.72^{c} ± 10.70	434.10^{c} ± 3.50	434.41^{c} ± 16.76
ADG	539.94^{a} ± 7.62	570.22^{b} ± 6.84	594.88^{c} ± 12.87	590.16^{c} ± 9.17	593.83^{c} ± 18.69
Survival	79.99^{a} ± 11.55	82.22^{b} ± 10.18	93.33^{c} ± 6.66	91.11^{c} ± 7.69	91.11^{c} ± 3.84
SGR	2.67^{a} ± 0.02	2.74^{b} ± 0.03	2.80^{c} ± 0.10	2.81^{c} ± 0.11	2.83^{c} ± 0.14
DGC	3.08^{a} ± 0.32	3.09^{a} ± 0.34	3.15^{b} ± 0.02	3.16^{b} ± 0.40	3.12^{b} ± 0.36
FCR	1.83^{a} ± 0.01	1.81^{a} ± 0.03	1.67^{c} ± 0.02	1.72^{b} ± 0.30	1.76^{b} ± 0.01

*Non-significant ($P > 0.05$).

All values are mean ± SE of three observations.

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

IBW (g), initial body weight; FBW (g), final body weight; WG (g per fish), weight gain = FBW (g) − IBW (g); WG (%), weight gain = (FBW − IBW/IBW) × 100; ADG (mg day^{-1}), average daily gain = [(FBW − IBW)/60 days] × 1000; Survival (%) = (final count of fish/initial count of fish) × 100; SGR (% day^{-1}), specific growth rate = [(ln FBW − ln IBW)/60 days] × 100; DGC (% day^{-1}), daily growth coefficient = [$(FBW^{1/3} - IBW^{1/3})$/60 days] × 100; FCR, feed conversion ratio = feed consumed (g, dry weight)/weight gain (g).

non-significant with the treatment groups fed diet containing 1.5 and 2% MOS supplementation.

Whole body composition and biological indices

Whole body composition of seabass fed the experimental diets is presented in (Table 3). Analysis of whole body composition of post-fed experimental animals revealed that there was no significant ($P > 0.05$) difference in moisture, crude protein, crude lipid, crude fibre and total ash contents among different treatments. The biological indices of seabass fed experimental diets supplemented with varying levels of MOS are presented in (Table 4). The CF showed that there was a tendency for an increased CF as the level of supplementation of MOS increases. The group fed 1.5% and 2% MOS-supplemented diet showed sig-

Table 3 Whole body composition (% Dry matter basis) of seabass fed experimental diets supplemented with varying levels of MOS for 60 days

Parameters	MOS 0%	MOS 0.5%	MOS 1%	MOS 1.5%	MOS 2%
Moisture	69.32 ± 0.07	69.35 ± 0.11	69.39 ± 0.24	69.28 ± 0.21	69.39 ± 0.06
Crude protein	63.21 ± 0.07	63.23 ± 0.05	63.38 ± 0.17	63.36 ± 0.32	63.34 ± 0.12
Crude lipid	15.90 ± 0.03	15.92 ± 0.06	15.89 ± 0.09	15.96 ± 0.04	15.88 ± 0.11
Crude fibre	0.72 ± 0.10	0.77 ± 0.05	0.75 ± 0.08	0.79 ± 0.10	0.77 ± 0.44
Total ash	20.46 ± 0.05	20.57 ± 0.14	20.49 ± 0.05	20.54 ± 0.07	20.47 ± 0.05

All values are mean ± SE of three observation.

Table 4 Biological indices of seabass fed experimental diets supplemented with varying levels of MOS for 60 days

Parameters	MOS 0%	MOS 0.5%	MOS 1%	MOS 1.5%	MOS 2%
CF	1.14^{a} ± 0.03	1.18^{ab} ± 0.05	1.27^{ab} ± 0.10	1.34^{b} ± 0.03	1.36^{b} ± 0.19
HSI	0.83^{a} ± 0.02	0.94^{b} ± 0.04	1.14^{c} ± 0.02	1.15^{c} ± 0.02	1.16^{c} ± 0.02
VSI	6.12^{a} ± 0.02	7.19^{e} ± 0.06	6.89^{d} ± 0.03	6.29^{b} ± 0.08	6.70^{c} ± 0.16

All values are mean ± SE of three observations.

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

CF (g cm^{-3}), condition factor = [(live weight, g)/$(length, cm)^{3}$] × 100.

HSI (%), hepatosomatic index = (liver weight, g/body weight, g) × 100.

VSI (%), viscerosomatic index = (visceral weight, g/body weight, g) × 100.

nificantly improved CF than the rest. Similarly, groups fed diets containing MOS showed significantly ($P < 0.05$) better hepatosomatic index (HSI) and viscerosomatic index (VSI) than the control diet without MOS supplementation.

Haematological and biochemical analysis

Haematological parameters of seabass fingerlings fed different levels of dietary MOS supplementation are presented in (Table 5). Results of the haematological parameters revealed that RBC, WBC, PCV, MCV, MCH and MCHC values were not significantly ($P > 0.05$) affected by dietary supplementation of MOS. However, significantly higher Hb content was observed in the fish fed 1% MOS-supplemented diet compared to the other treatments. Biochemical parameters of seabass fingerlings fed different levels of dietary MOS are given in (Table 6). No significant differences were observed between treatments for total protein, albumin, globulin, ALT, AST, ALP and LDH. However, the glucose, urea, cholesterol and triglyceride showed significantly higher values in 2% MOS-supplemented diet compared to control.

Histological examination

The effect of supplementation of MOS on the histological changes in the intestine and liver is depicted in (Fig. 1). Histological studies on the post-fed experimental animals revealed that the intestine of fish fed control diet 0% MOS appeared normal with most of the villi appearing straight. The intestinal layers also appeared normal without any histological changes. However, MOS

Table 5 Haematological parameters of seabass fed experimental diets supplemented with varying levels of MOS for 60 days

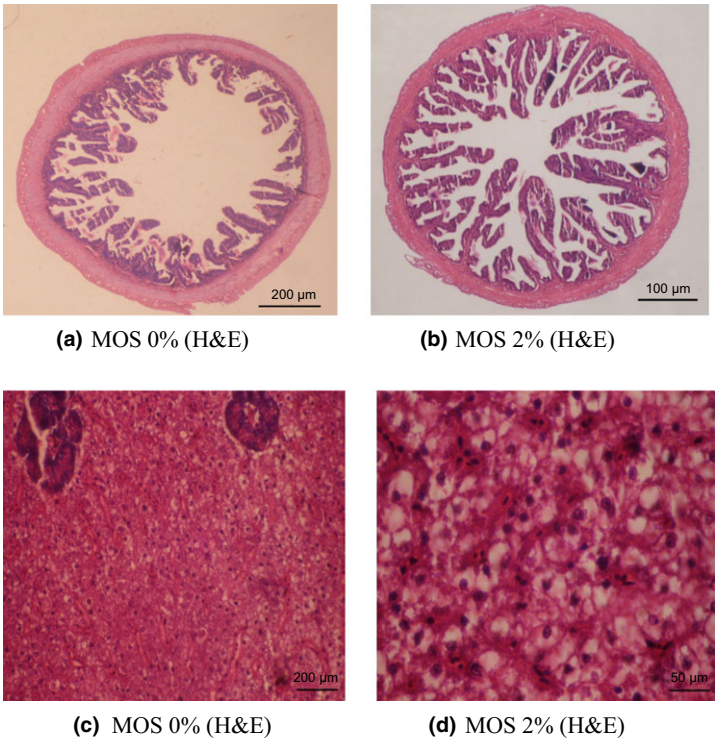
Parameters	MOS 0%	MOS 0.5%	MOS 1%	MOS 1.5%	MOS 2%
RBC (10^6/mm^{-3})	3.34 ± 0.51	3.28 ± 0.38	3.40 ± 0.48	3.30 ± 0.46	3.36 ± 0.21
WBC (10^3 mm^{-3})	8.28 ± 0.61	8.21 ± 0.58	8.28 ± 0.52	8.26 ± 0.51	8.24 ± 0.47
Hb (g dL^{-1})	7.25^{a} ± 0.03	8.15^{b} ± 0.06	8.61^{e} ± 0.01	8.26^{c} ± 0.06	8.44^{d} ± 0.04
PCV (%)	33.23 ± 2.65	34.81 ± 4.04	38.29 ± 3.16	35.72 ± 4.45	32.50 ± 2.62
MCV (fl)	79.73 ± 1.40	81.44 ± 4.51	84.15 ± 5.32	83.82 ± 4.97	76.30 ± 4.20
MCH (pg)	24.8 ± 3.92	22.68 ± 1.84	22.75 ± 1.95	27.63 ± 5.69	24.50 ± 3.50
MCHC (g dL^{-1})	30.12 ± 2.27	27.17 ± 2.79	27.50 ± 3.29	24.18 ± 5.31	30.53 ± 1.26

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 6 Biochemical parameters of seabass fed experimental diets supplemented with varying levels of MOS for 60 days

Parameters	MOS 0%	MOS 0.5%	MOS 1%	MOS 1.5%	MOS 2%
Glucose (mg dL^{-1})	100.10^{a} ± 5.81	124.43^{bc} ± 5.63	133.13^{c} ± 4.74	118.23^{b} ± 7.13	130.53^{c} ± 5.25
Urea (mg dL^{-1})	4.39^{a} ± 0.98	3.91^{a} ± 1.00	3.66^{a} ± 0.40	15.36^{b} ± 4.55	16.16^{b} ± 4.83
Cholesterol (mg dL^{-1})	246.03^{a} ± 5.31	254.66^{a} ± 5.60	259.16^{a} ± 9.95	312.23^{b} ± 4.68	320.26^{b} ± 8.36
Triglyceride (mg dL^{-1})	208.40^{a} ± 6.50	218.43^{a} ± 9.80	248.16^{b} ± 9.70	238.03^{b} ± 7.84	324.53^{c} ± 5.31
Total Protein (g dL^{-1})	1.32 ± 0.59	1.23 ± 0.15	1.3 ± 0.26	0.85 ± 0.03	0.84 ± 0.03
Albumin (g dL^{-1})	0.55^{b} ± 0.04	0.53^{b} ± 0.02	1.06^{c} ± 0.02	0.46^{a} ± 0.03	0.45^{a} ± 0.03
Globulin (g dL^{-1})	0.76 ± 0.60	0.70 ± 0.14	0.69 ± 0.22	0.38 ± 0.06	0.34 ± 0.04
AST (U L^{-1})	76.23^{c} ± 5.67	38.13^{a} ± 4.60	70.10^{c} ± 4.20	48.56^{b} ± 4.40	26.66^{a} ± 10.15
ALT (U L^{-1})	33.19^{a} ± 4.11	26.13^{a} ± 0.59	63.86^{b} ± 5.48	27.13^{a} ± 2.99	30.76^{a} ± 8.31
ALP (U L^{-1})	31.06^{a} ± 6.04	27.43^{a} ± 2.60	64.46^{b} ± 6.46	32.43^{a} ± 9.66	25.5^{a} ± 0.36
LDH (U L^{-1})	1076.00 ± 17.52	1040 ± 23.16	1067.33 ± 29.02	1057.33 ± 19.04	1044.76 ± 31.67

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.



(a) MOS 0% (H&E) **(b)** MOS 2% (H&E)

(c) MOS 0% (H&E) **(d)** MOS 2% (H&E)

Figure 1 Light photomicrograph of *Lates calcarifer* intestine and liver sections with haematoxylin and eosin. (a) Intestine of fish fed control diet (b) Fish fed 2% MOS diet showing the positive effect of improved intestinal folding. (c) Liver section of fish fed control diet showing normal hepatocytes (d) Fish fed 2% MOS diet liver showing vacuolated hepatocytes.

supplementation resulted in broadening of few villi with increase in number of secretory vacuoles (goblet cells) especially in the upper third portion of the villi. Each villus had additional side branches indicating the hyperplasia in the villus epithelium which were seen on both the sides of the villus thereby it could increase the absorptive surface for nutrients. In addition, MOS supplementation showed the presence of more number of cells in lamina propria, mucosal layer as well as sub mucosal layer. The group fed 2% MOS-supplemented diet showed increased side branches of villi which occupied entire lumen of the intestine. Similarly, the hepatic tissue appeared normal in the control group. Supplementation of MOS at varying levels showed vacuolations in few hepatocytes with centrally placed nuclei. Sinusoidal space found to be increased with presence of erythrocytes; few hepatocytes appeared enlarged and had intensive vacuolations resembling glycogen deposits. The histological changes were more evident in the diet with higher supplementation of MOS.

Discussion

This was the first study to investigate the effect of MOS on growth, body composition, haematology, biochemical parameters and histology of *L. calcarifer*. Growth performance of seabass fed MOS-containing diets indicated that MOS supplementation had a beneficial effect in the diet of seabass. Fish fed the diet containing 1% MOS showed improved growth rate, ADG, DGC, SGR, Survival and FCR. Better survival was recorded in fish fed diets supplemented with MOS at more than 1% level. The improved growth performance and survival of fish for MOS supplementation has been reported by earlier workers (Torrecillas, Makol, Caballero, Montero, Robaina, Real, Sweetman, Tort & Izquierdo 2007; Yilmaz, Genc & Genc 2007; Grisdale-Helland, Helland & Gatlin 2008; Gultepe, Salnur, Hossu & Hisar 2010; Ye, Wang, Li & Sun 2011; Ahmad, El-Mousallamy, Awad & Abd El-Naby 2013). On the contrary, several studies have showed that growth performance and survival remained un affected with MOS supplementation in various fish species (Pryor, Royes, Chapman & Miles 2003; Genc, Yilmaz, Genc & Aktas 2007; Welker, Lim, Yildirim-Aksoy, Shelby & Klesius 2007; Akrami, Karimabadi, Mohammadzadeh & Ahmadifar 2010; Dimitroglou, Merrifield, Spring, Sweetman, Moate & Davies 2010; Gultepe, Hisar, Salnur, Hossu, Tansel Tanrikul & Aydln 2012; Razeghi Mansour, Akrami, Ghobadi,

Amani, Ezatrahimi & Gharaei 2012; Vellaichamy, Kaliyan, Thangappan & Muthusamy 2013; Talpur, Munir, Anna Mary & Hashim 2014). The beneficial effect may be attributed to the potential prebiotic effect of efficiently discriminating and eliminating the pathogenic organisms, improving the *in vivo* digestion and absorption in 1% MOS-supplemented diet (Safari, Shahsavani, Paolucci & Atash 2014).

The whole body composition of seabass fed experimental diets containing varying levels of MOS showed non-significant differences among the treatment groups. Similar to our findings, Dimitroglou *et al.* (2010) and Gultepe *et al.* (2010) on gilthead sea bream (*Sparus aurata*) reported that 2 and 4 g kg^{-1} MOS supplementation had no significant difference in whole body composition. On the contrary, studies on rainbow trout (*Oncorhynchus mykiss*) and hybrid tilapia (*Oreochromis niloticus* × *O. aureus*), reported increased protein concentration as the level of MOS was increased in the diet from 1.5 to 4.5 g kg^{-1} (Genc *et al.* 2007; Yilmaz *et al.* 2007).The condition factor was highest in the group fed 1.5% MOS diet (1.34%) and it showed a linear increasing trend as the level of inclusion of MOS increased from 0% to 2%. Condition factor is used to compare the 'condition', 'fatness' or 'well being' of fish and are based on the hypothesis that heavier fish of a given length are in better condition. Ta'ati *et al.* (2011) reported that CF was significantly affected by a prebiotic immumoster at 3% compared to 1% and the control diet without any prebiotic. The HSI ranged from a lower value of 0.83% in MOS 0% diet to a higher value of 1.16% in MOS 2% diet and the differences are significant among the experimental diets supplemented with varying levels of MOS. The VSI ranged from a lower value of 6.12% in MOS 0% diet to the highest value of 7.19% in MOS 0.5% diet and the differences are significant among the experimental diets supplemented with varying levels of MOS. Genc *et al.* (2007) reported non-significant difference in VSI in hybrid tilapia fed diets containing MOS 0–4.5 g kg^{-1}.

The haematological parameters were not significantly affected by the dietary MOS supplementation in this study. The fish fed 1% MOS showed significantly higher Hb, than the rest of the treatments. Welker *et al.* (2007) reported that inclusion of MOS had no effect on haematological parameters (RBC, WBC, PCV and Hb) of Channel catfish (*Ictalurus punctatus*). However, Andrews, Sahu, Pal and Kumar (2009) observed a significant difference in WBC, RBC, Hb and MCV in Rohu (*Labeo rohita*) fed on the MOS-supplemented diet against the control diet without MOS supplementation. The variation in the results of different blood parameters could be attributed to the prebiotic type, dose and other wide spectrum of factors. 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 (Osuigwe, Obiekezie & Onuoha 2005).Currently, there are few data available on the prebiotic effect on the blood indicators of seabass. The present study showed that there was as significant increase in glucose, urea, cholesterol and triglyceride content in fish fed 2% MOS diet, whereas no significant differences were observed in the other biochemical parameters among the various treatments. Razeghi Mansour *et al.* (2012) reported that dietary MOS had no effect in blood serum, glucose, cholesterol, triglyceride, protein, urea and albumin of beluga (*H. huso*) juveniles, after 46-day feeding on diets containing two levels of prebiotic 2 and 4 g kg^{-1} MOS. In contrast, Andrews *et al.* (2009) observed a significant improvement in serum protein and albumin in (*L. rohita*) fed on the MOS supplemented diet in comparison to control. The increase in the serum protein and albumin levels is considered to be associated with a stronger innate response in fish (Andrews *et al.* 2009). Ye *et al.* (2011) also observed that Japanese flounder (*Paralichthys olivaceus*) supplemented with prebiotics showed no significant differences in the concentration of cholesterol and triglyceride after an 8-week test period. It appears that fluctuations in haematological and biochemical variables may be species-specific and depend on the inclusion rates of MOS, diet ingredients and the rearing period (Ta'ati *et al.* 2011). In the present study, the amount of blood glucose was 100–133 mg dL^{-1} and the highest value was observed in the group receiving MOS 1%. In the present study, serum LDH, AST, ALT and ALP levels were not affected by dietary MOS supplementation. Similar to our results Akrami, Razeghi Mansour, Ghobadi, Ahmadifar, Shaker Khoshroudi and Moghimi Haji (2013) reported that activities of LDH, AST, ALT and ALP levels were not affected by dietary MOS. The serum activity of ALT and AST obviously varies depending on fish species.

Histological analysis revealed that MOS supplementation resulted in increased microvilli density in the anterior and posterior intestinal regions. The analysis suggests that MOS 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 MOS 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 MOS 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. Dietary Bio-Mos supplementation at 0.4% for an 8-week period in European seabass showed increased gut mucosal folds surface through fold height and width in anterior and posterior gut (Torrecillas, Makol, Benítez-Santana, Caballero, Montero & Sweetman 2011).The beneficial effect of Bio-Mos supplementation up to 0.4% for 9 weeks was also reported in gilthead sea bream (*S. aurata*) Dimitroglou *et al.* (2010). Red drum (10.9 g) fed 1% Bio-Mos for 4 weeks presented longer folds and microvilli Anguiano, Pohlenz, Buentello and Gatlin (2013), but no effect was reported in posterior gut when supplemented for 8 weeks to smaller fish (7 g). Gulf of Mexico sturgeon (*Acypenser oxyrinhus*) fed 0.3% MOS (product not specified) did not present any gut morphological change in terms of spiral valve villi length, width and density Pryor *et al.*(2003).Torrecillas *et al.* (2007) reported that 0.4% MOS supplementation did not increase European sea bass intestinal villi length. The differences between the reported effects of MOS on villi structure to date might be due to the different doses used, different species assessed different gut microbiota within these species, different rearing conditions or different methodological approaches used. An improvement in intestinal morphology is not only likely to benefit feed utilization, but the maintenance of an intact, healthy mucosal epithelium reduces the chances of opportunistic indigenous bacterial infections. Indeed, this may well have been a contributory factor to the improved growth performance, and survival obtained in the current study.

Results from this study showed that MOS supplementation alters the morphological characters of the intestine and has got beneficial effect on growth and survival of seabass. Further it can be concluded that inclusion of MOS in the range of 1–1.5% levels in the diet of seabass would be beneficial. However, further studies on microbial diversity and immunological parameters would help in conclusively confirming the prebiotic effect of MOS.

Acknowledgments

The authors are grateful to the Indian Council of Agricultural Research, New Delhi for the project on Outreach activity on Fish feeds. Authors express their sincere thanks to Dr. A.G. Ponniah, former Director and Dr. K.K. Vijayan, Director, Central Institute of Brackishwater Aquaculture for providing necessary facilities for carrying out this work.

References

Ahmad M.H., El-Mousallamy A., Awad S.M.M. & Abd El-Naby A.S. (2013) Evaluation of Bio-Mos as a feed additive on growth performance, physiological and immune responses of Nile tilapia (*Oreochromis niloticus* L.). *Journal of Applied Science and Research* **9**, 6441–6449.

Akrami R., Karimabadi A., Mohammadzadeh H. & Ahmadifar E. (2010) Effect of dietary mannan oligosaccharide on growth performance, survival, body composition and salinity stress resistance in Kutum (*Rutilus frisii kutum*) fry stage. *Journal of Marine Science and Technology* **8**, 47–57.

Akrami R., Razeghi Mansour M., Ghobadi S.H., Ahmadifar E., Shaker Khoshroudi M. & Moghimi Haji M.S. (2013) Effect of prebiotic mannan oligosaccharide on hematological and blood serum biochemical parameters of cultured juvenile great sturgeon (*Huso huso* Linnaeus, 1754). *Journal of Applied Ichthyology* **29**, 1–5.

Andrews S.R., Sahu N.P., Pal A.K. & Kumar S. (2009) Hematological modulation and growth of *Labeo rohita* fingerlings: effect of dietary mannan oligosaccharide, yeast extract, protein hydrolysate and chlorella. *Aquaculture Research* **41**, 61–69.

Anguiano M., Pohlenz C., Buentello A. & Gatlin D.M. III (2013) The effects of prebiotics on the digestive enzymes and gut histomorphology of red drum (*Sciaenops ocellatus*) and hybrid striped bass (*M. chrysops* × *M. saxatilis*). *British Journal of Nutrition* **109**, 623–629.

Anon (1984) Sigma diagnostics TM Lactic dehydrogenase (quantitative, colorimetric determination in serum, urine and cerebrospinal fluid) at 400-450 nm. Procedure No. 500, Sigma Chemical Company, St. Louis, MO, USA.

AOAC (Association of Official Analytical Chemists) (2012) *Official Methods of Analysis of the Association of Official Analytical Chemists* (19th edn). Association of Official Analytical Chemists, Arlington, VA, USA.

Blaxhall P.C. & Daisley K.W. (1973) Routine hematological methods for use with fish blood. *Journal of Fish Biology* **5**, 771–781.

Borges A., Scotti L.V., Siqueira D.R., Jurinitz D.F. & Wassermann G.F. (2004) Hematological and serum biochemical values for jundia (*Rhamdia quelen*). *Fish Physiology and Biochemistry* **30**, 21–25.

Dimitroglou A., Merrifield D.L., Spring P., Sweetman J., Moate R. & Davies S. (2010) Effect of mannan oligosaccharide (MOS) supplementation on growth performance feed utilization, intestinal histology and gut microbiota of gilthead sea bream (*Sparus aurata*). *Aquaculture* **300**, 182–188.

England J.M., Walford D.M., Waters D.A. (1972) Reassessment of the reliability of hematocrit. *British Journal of hematology* **23**, 247–253.

Fawcett J.K. & Scott J.E. (1960) A rapid and precise method for the determination of urea. *Journal of Clinical Pathology* **13**, 156–159.

Feldman B.F., Zinkl J.G. & Jian N.C. (2000) *Schalm's Veterinary Hematology*, pp. 1120–1125. Lippincott, Williams and Wilkins Publication, Baltimore, Canada.

Gatesoupe F.J. (1999) The use of probiotics in aquaculture. *Aquaculture* **180**, 147–165.

Genc M.A., Yilmaz E., Genc E. & Aktas M. (2007) Effect of dietary mannan oligosaccharides (MOS) on growth, body composition, intestine and liver histology of the hybrid tilapia (*Oreochromis niloticus* × *O. aureus*). *Israeli Journal of Aquaculture* **59**, 10–16.

Glencross B.D. (2006) Nutritional management of barramundi (*Lates calcarifier*) - a review. *Aquaculture Nutrition* **12**, 291–309.

Grisdale-Helland B., Helland S.J. & Gatlin D.M. III (2008) The effects of dietary supplementation with mannan oligosaccharide fructooligosaccharide or galacto oligosaccharide on the growth and feed utilization of Atlantic salmon (*Salmo salar*). *Aquaculture* **283**, 163–167.

Gultepe N., Salnur S., Hossu B. & Hisar O. (2010) Dietary supplementation with Mannan oligosaccharides (MOS) from Bio-Mos enhances growth parameters and digestive capacity of gilthead sea bream (*Sparus aurata*). *Aquaculture Nutrition* **17**, 482–487.

Gultepe N., Hisar O., Salnur S., Hossu B., Tansel Tanrikul T. & Aydln S. (2012) Preliminary assessment of dietary mannan oligosaccharides on growth performance and health status of gilthead seabream (*Sparus auratus*). *Journal of Aquatic Animal Health* **24**, 37–42.

Lowry H.W., Rosebrough N.J., Farr A.L. & Randall R.J. (1951) Protein measurement with folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.

Manning T.S. & Gibson G.R. (2004) Prebiotics. *Best Practice and Research Clinical Gastroenterology* **18**, 287–298.

Nandakumar S., Ambasankar K., Syamadayal J., Raman C. & Ali S.R. (2013) Fish meal replacement with chicken waste meal in Asian seabass (*Lates calcarifer*) feeds. *Indian Journal of Fisheries* **60**, 109–114.

Osuigwe D.I., Obiekezie A.I. & Onuoha G.C. (2005) Some hematological changes in hybrid catfish (*Heterobranchus longifilis* × *Clarias gariepinus*) fed different dietary levels of raw and boiled jack bean (*Canavalia ensiformis*) seed meal. *African Journal of Biotechnology* **4**, 1017–1021.

Parekh A.C. & Jung D.H. (1970) Cholesterol determination with ferric acetate- 348 uranyl acetate sulphuric acid, ferrous sulphate reagents. *Analytical Chemistry* **42**, 1423–1427.

Pryor G.S., Royes J.B., Chapman F.A. & Miles R.D. (2003) Mannan oligosaccharides in fish nutrition: effects of dietary supplementation on growth and gastrointestinal villi structure in Gulf of Mexico sturgeon. *North American Journal of Aquaculture* **65**, 106–111.

Razeghi Mansour M., Akrami R., Ghobadi S.H., Amani Denji.K., Ezatrahimi N. & Gharaei A. (2012) Effect of dietary mannan oligosaccharide (MOS) on growth performance, survival, body composition, and some hematological parameters in giant sturgeon juvenile (Huso huso) Linnaeus, 1754. *Fish Physiology and Biochemistry* **38**, 829–835.

Reitman S. & Frankel S. (1957) A colorimetric method for the determination of serum glutamate oxaloacetate and glutamate pyruvate transaminases. *American Journal of Clinical Pathology* **28**, 56–63.

Rice E.C. (1970) *Triglycerides in Serum: Standard Methods of Clinical Chemistry*, Vol. VI (ed. by P. Ceds Roberict), pp. 215–222. Medorald Academic Press, New York, USA.

Ringo E., Olsen R.E., Gifstad T.O., Dalmo R.A. & Amhund H. (2010) Prebiotics in aquaculture: a review. *Aquaculture Nutrition* **16**, 117–136.

Roberts R.J. (2001) *Fish Pathology* (3rd edn), pp. 380–386. W. B. Saunders, London, UK.

Safari O., Shahsavani D., Paolucci M. & Atash M.M.S. (2014) Single or combined effects of fructo- and mannan oligosaccharide supplements on the growth performance, nutrient digestibility, immune responses and stress resistance of juvenile narrow clawed crayfish (*Astacus leptodactylus leptodactylus*) Eschscholtz, 1823. *Aquaculture* **432**, 192–203.

Ta'ati R., Soltani M., Bahmani M. & Zamini A.A. (2011) Growth performance, carcass composition and immune physiological indices in juvenile great sturgeon (*Huso huso*) fed on commercial prebiotic, Immunoster. *Iranian Journal of Fishery Science* **10**, 324–335.

Talpur A.D., Munir M.B., Anna Mary A. & Hashim R. (2014) Dietary probiotics and prebiotics improved food acceptability, growth performance, hematology and

immunological parameters and disease resistance against *Aeromonas hydrophila* in snakehead (*Channa striata*) fingerlings. *Aquaculture* **426–427**, 14–20.

Torrecillas S., Makol A., Caballero M.J., Montero D., Robaina L., Real F., Sweetman J., Tort L. & Izquierdo M.S. (2007) Immune stimulation and improved infection resistance in European seabass (*Dicentrarchus labrax*) fed mannan oligosaccharides. *Fish and Shell fish Immunology* **23**, 969–981.

Torrecillas S., Makol A., Benítez-Santana T., Caballero M.J., Montero D. & Sweetman J. (2011) Reduced gut bacterial translocation in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides (MOS). *Fish and Shellfish Immunology* **30**, 674–681.

Trinder P. (1969) Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry* **6**, 24–27.

Vellaichamy R., Kaliyan M., Thangappan A. & Muthusamy T. (2013) The efficacy of dietary yeast mannan oligosaccharide on growth and survival rate in (*Amphiprion ocellaris*) fingerlings. *European Journal of Biotechnology and Bioscience* **1**, 12–15.

Welker T.L., Lim C., Yildirim-Aksoy M., Shelby R. & Klesius P.H. (2007) Immune responseand resistance to stress and *Edwardsiella ictaluri*, fed diets containing commercial whole cell yeast or yeast subcomponents. *Journal of the World Aquaculture Society* **38**, 24–35.

Wintrobe M.M. (1974) *Clinical Hematology* (7th edn), pp. 125. Lea and Febiger, Philadelphia, PA, USA.

Ye J.D., Wang K., Li F.D. & Sun Y.Z. (2011) Single or combined effects of fructo- and mannan oligosaccharide supplements and *Bacillus clausii* on the growth, feed utilization, body composition, digestive enzyme activity, innate immune response and lipid metabolism of the Japanese flounder (*Paralichthys olivaceus*). *Aquaculture Nutrition* **17**, 902–911.

Yilmaz E., Genc M.A. & Genc E. (2007) Effects of dietary mannan oligosaccharides on growth, body composition, intestine and liver histology of rainbow trout (*Oncorhynchus mykiss*). *Israeli Journal of Aquaculture* **59**, 182–188.

