Effect of dietary mannanoligosaccharide on intestinal micro biota and immune parameters of Asian seabass (*Lates calcarifer*) juveniles.

S Syed Raffic Aliab*, K Ambasankara, P Ezhil Praveenaa

Department of Zoology, ICAR-Central Institute of Brackish Water Aquaculture, Tamil Nadu, India

Abstract

A sixty days feeding experiment was carried out to evaluate effect of dietary Mannanoligosaccharide (MOS) on gut micro biota and immune parameters of Asian sea bass (*Lates calcarifer*) juveniles with mean initial body weight of (8.13 ± 0.06 g) were allocated in to 15 tanks with three replicates for each treatment with each replicate containing 15 animals. Experimental diets were prepared to contain (40% protein and 8% lipid) by supplementing MOS at four different concentrations (control 0%, 0.5%, 1%, 1.5% and 2%) levels in the diet of Asian sea bass. At the end of the experiment the results of serum immune parameters showed significant ($p<0.05$) differences in lysozyme activity, Alternative complement pathway activity, superoxide dismutase and nitro blue tetrazolium assay between control and MOS supplemented diets. PCR and DGGE analysis revealed that intestinal samples from MOS supplemented diet remain unaffected was failed to increase the intestinal microbial diversity and species richness. The results revealed that dietary MOS at 1.5% to 2% exerted a greater effect in immune parameters.

Keywords: Asian sea bass, Immune parameters, Mannanoligosaccharide, Micro biota.

Accepted on 03rd September, 2021

Introduction

Asian seabass *Lates calcarifer* (Bloch) popularly called as barramundi is a carnivorous, euryhaline, commercially valuable food fish widely cultured in tropical and subtropical regions of Asia and Pacific countries in marine, brackish and fresh water resources [1]. Because of its high market value and excellent growth potential sea bass is considered as an alternate candidate species in brackish water aquaculture in India [2]. Bacterial and viral diseases are the major outbreaks in aquatic organisms causing considerable economic losses in aquaculture industry [3]. Antibiotics and chemo-therapeutics are the most commonly used therapeutic agents to prevent/control these aquatic diseases, such as suppression of aquatic animal's immune system, drug resistant, food safety problems etc [4]. As an alternatives for the antibiotics, use of prebiotics such as Arabinoxylooligosaccharides (AXOS), Fructooligosaccharides (FOS), Galactooligosaccharides (GOS), Isomaltooligosaccharides (IMO), inulin and Mannanoligosaccharides (MOS), and Xylooligo-saccharides (XOS), has been effective to disease control, immune system activation, and promoting the growth and health of aquatic organisms [5].

Mannanoligosaccharides is one of the prebiotics widely used in various fish species to modulate growth performance, survival and immune-related parameters [6-8]. MOS is composed of complex carbohydrate molecules, derived from the outer cell wall of the yeast Saccharomyces cerevisiae, whose main components are β-glucans (mannoproteins) are known as elements capable of activating the immune systems of animals [9]. The mode of action of MOS is based in two main functions blocking pathogen colonization and modulating immune system [10]. Several studies have showed the benefits of prebiotics on the growth performance, survival, physiological status, digestive enzyme activities, and immune response etc. [1,11,12]. Therefore the current study was investigated to evaluate the effect of MOS on intestinal micro biota and immune parameters in the diet of Asian sea bass juveniles.

Materials and Methods

Preparation of experimental diets

The experimental diets used in the present study were formulated to contain approximately 40% protein and 8% lipid, which is sufficient for the optimal growth of Asian sea bass juveniles. Diets were prepared by supplementing MOS in the standard sea bass diet at four different concentrations at 0%, 0.5%, 1%, 1.5% and 2% levels following the methods of MOS (mannanoligosaccharide) study. The proximate composition of the ingredients and experimental diets (Tables 1 and 2) were analyzed by following standard procedures of MOS [13].

Table 1. *Ingredient composition (%) of experimental diets containing varying levels of MOS.*

Diets/ Ingredients (%)	MOS 0%	MOS 0.5%	MOS 1%	MOS 1.5%	MOS 2%
Fish meal[1]	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[1]	4	4	4	4	4
Lecithin	1	1	1	1	1
Vitamin and Minerals[2]	3	3	3	3	3
Binder[3]	1	1	1	1	1
Cellulose	2	1.5	1	0.5	0
MOS[4]	0	0.5	1	1.5	2

[1]Sardine fishmeal and fish oil. Bismifisheries, Mayiladuthurai, Tamil Nadu, India.
[2]Commercially sourced premix and each kg contains Vitamin A-2000000IU, Vitamin D-400000 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.
[3]Pegabind, BentoliAgri nutrition Asia pvt Ltd, Singapore.
[4]MOS Alltech,-Bangalore.

Table 2. *Proximate composition (%) of experimental diets containing varying levels of MOS.*

Diets	MOS 0%	MOS 0.5%	MOS 1%	MOS 1.5%	MOS 2%
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 fiber	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

Feeding trial

Asian sea bass juveniles (average body weight of 8.0 g) were obtained from the Kuluthumedu village from the (Women self-help groups Ponneri, Chennai) were randomly allocated into 15 tanks (1000 L) at a density of 15 fish per tank (3 tanks per treatment). The tanks were supplied with sand-filtered seawater with continuous aeration through air diffuser stones. During the experimental trial, which lasted for 60 days the fishes were hand fed twice a day (morning 9.00 and evening 15.00 hrs). The fecal matter was removed daily before feeding. In Table 3 water quality parameters pH, temperature, salinity, dissolved oxygen and total ammonia nitrogen was analyzed as per the standard procedures of American Public Health Association, 1998. [14].

Table 3. *Water quality parameters.*

S.No	Water parameters	Values
1	pH	7.2-8.1
2	Temperature	26ºC-28ºC
3	Salinity	28-35 g L^{-1}
4	Dissolved oxygen	6.0-7.3 mg L^{-1}
5	Total ammonia nitrogen	0.08-0.11 mg L^{-1}

Blood sampling

At the end of the feeding experiment, fishes were starved for 24 h before sampling. Three fish from each replicate were randomly collected and anaesthetized (2-phenoxyethanol at 0.5 ml/L) for blood sample collection [1].

Serum immune parameters

The serum immune parameters lysozyme activity, Alternative complement pathway activity, superoxide dismutase assay and nitroblue tetrazolium assay was carried out following the protocols of Ali SR, et al. research work [1].

PCR-DGGE analysis and sequencing

DNA extraction, Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) and sequencing analysis was performed as described in Table 4 [15-17].

Table 4. *16S rRNA primers used in this study.*

Primer	Sequence (5' to 3')	Reference
357f	CCTACGGGAGGCAGCAG	Muyzer, et al.
357f-GC	GCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGG GGGGCCTACGGGAGGCAGCAG	
907rM	CGTCAATTCMTTTGAGTTT	

Statistical analysis

Data were subjected for statistical analysis using one-way ANOVA and the means were compared using Duncan's multiple range test. Mean values were considered significantly different at $p<0.05$. Statistical analysis was performed with SPSS statistical package ver.17.0 (SPSS, Chicago, USA).

Results

The serum immune parameters lysozyme, ACP, SOD and NBT assay of Asian sea bass fed diets supplemented with FOS showed significant ($p<0.05$) differences between control and treatment groups. Highest lysozyme, ACP, SOD and NBT activity was observed in fish fed with 1.5% and 2% MOS supplemented diet compared various treatments (Figures 1-6).

Denaturing Gradient Gel Electrophoresis (DGGE) of bacterial 16S *rDNA* amplicons from Asian sea bass intestinal contents obtained in trial is shown in Figure 6. The Polymerase chain reaction PCR and DGGE analysis of gut samples of sea bass fed with different levels of MOS supplemented diets was found to fail to increase the bacterial diversity.

Figure 1. *Fish rearing and experimental design.*

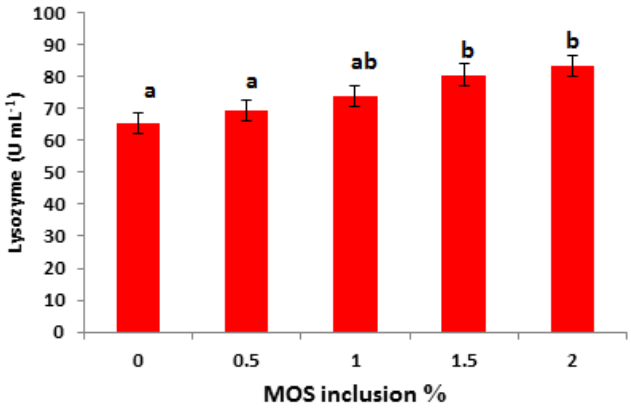

Figure 2. *Lysozyme activity of seabass fed experimental diets supplemented with varying levels of MOS for 60 days (Mean ± SD) (n=3). Bars assigned with different superscripts are significantly different (p<0.05).*

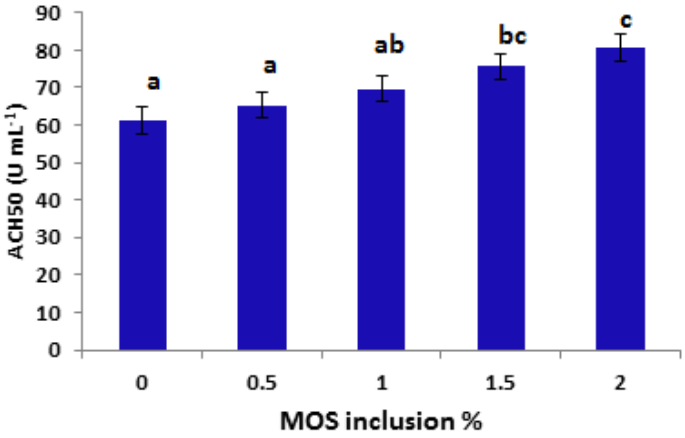

Figure 3. *ACP activity of seabass fed experimental diets supplemented with varying levels of MOS for 60 days (Mean ± SD) (n=3). Bars assigned with different superscripts are significantly different (p<0.05).*

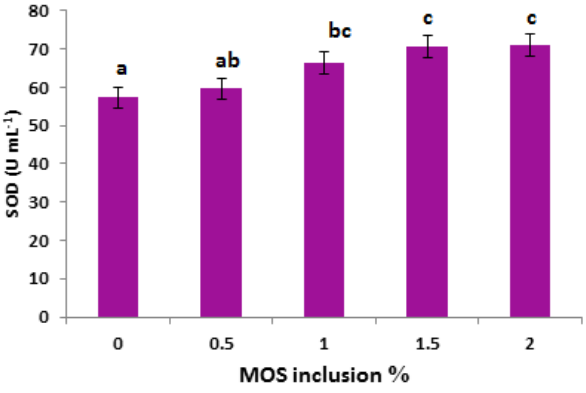

Figure 4. *SOD activity of seabass fed experimental diets supplemented with varying levels of MOS for 60 days (Mean ± SD) (n=3). Bars assigned with different superscripts are significantly different (p<0.05).*

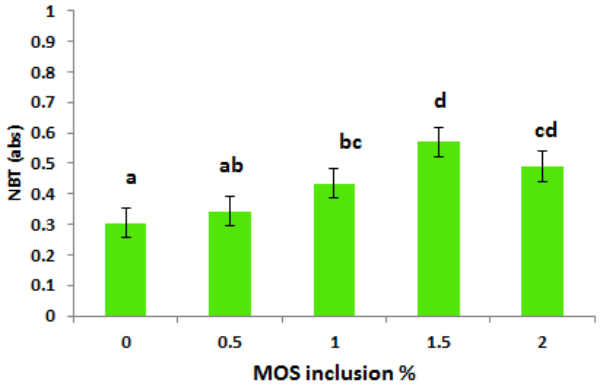

Figure 5. *NBT assay of seabass fed experimental diets supplemented with varying levels of MOS for 60 days (Mean ± SE) (n=3). Bars assigned with different superscripts are significantly different (p<0.05).*

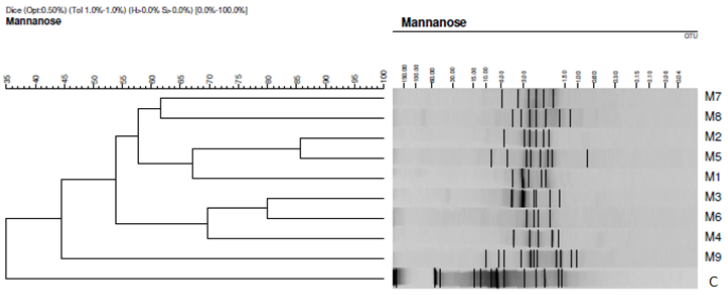

Figure 6. *Effect of MOS on the gut microbial community in the intestinal contents of seabass as evaluated by denaturing gradient gel electrophoresis of bacterial 16S r DNA amplicons. C and M1=control diet (0%) MOS, M 2 and M3=(0.5%) MOS, M4 and M5=(1%) MOS, M6 and M7=1.5%) MOS, M8 and M9=(2%) MOS.*

Discussion

This is the first study to report the efficacy of MOS on gut microbiota and immunological parameters in Asian sea bass juveniles. The lysozyme activity of sea bass fed diets supplemented with MOS showed significant difference between control and treatment groups. The highest lysozyme activity was observed in fish fed with 2% MOS supplemented diet compared to various treatments. Similar to our results reported that *Cyprinus carpio* fed with 4.5 g kg^{-1} MOS supplemented diet had significantly higher serum lysozyme activity compared to the rest of the diets [6]. Dietary supplementation of prebiotics MOS in fish diets elevates the antibacterial activities, gut mucus and lysozyme and [18]. Lysozyme activity is an important index of innate immunity of fish and is ubiquitous in its distribution among living organisms, and it is an important enzyme in blood that actively lyses bacteria an increased levels has been considered to be a natural protective mechanism in fish [19]. ACP and SOD activity of sea bass fed with MOS supplemented diets showed significant differences between control and treatment groups. Higher ACP and SOD activity was recorded in fish fed with 2% MOS supplemented diet. Similarly reported that serum ACP was significantly higher in fish fed with 4.5 g kg^{-1} MOS compared to fish fed with control diet. Similarly reported that 0.1% and 0.2% MOS supplementation could significantly increase SOD activity in sea cucumber [20]. The complement system is essential for the innate immune system of the fish and is most well-known for its capacity to kill pathogens by creating pores in their surface membranes or by opsonizing pathogens by stimulating phagocytosis. The ACP activity is very active in fish serum when compared to mammals, this pathway is very important in the defence mechanism of fish [21]. Superoxide dismutase constitutes a very important antioxidant defense against oxidative stress in the body of fish [22]. This enzyme acts as a good therapeutic agent against reactive oxygen species-mediated disease [23]. The NBT assay sea bass fed with MOS supplemented diets showed significant difference between control and treatment groups. The fish fed with 1.5% MOS supplemented diet showed highest NBT compared to rest of the treatments. Similarly it was reported that in African catfish, fed with MOS supplemented diets showed increased NBT activity during the first two weeks and then decreased back to the control level after 45 days [24].

The effect of MOS supplementation on the intestinal microbial community of sea bass was analyzed using PCR-DGGE. MOS was found to fail to increase the bacterial diversity in sea bream reported that MOS can influence the intestinal microbiota in terms of microbial abundance and species richness and this positive effect in microbial diversity [25]. Previous studies have reported that the effect of dietary MOS on fish intestinal microbiota in rainbow trout juveniles fed with 0.2% MOS supplemented diets for 111 days showed decreased viable *Aeromonas/vibrio* sp, *Micrococcus* sp. and gram positive rod bacterial populations and increased *Enterococcus* sp. and *Enterobacteriaceae* levels. The same fish species fed with MOS supplemented diets for fifty eight days showed increase in *Pseudomonas* sp. levels and reduction in *Micrococcus* sp. [26].

Conclusion

The current study effect of dietary mannanoligosaccharide on intestinal micro biota and immune parameters of Asian seabass (*Lates calcarifer*) juveniles showed that MOS is a beneficial prebiotic supplement for improving the immunological parameters of Asian sea bass. However, further research is required to conclusively ascertain the prebiotic effect of MOS supplementation for enhancing the gut microbiota and disease resistance.

Acknowledgements

The Authors express sincere and profound sense of gratitude to Dr.K.K.Vijayan, Director, Central Institute of Brackishwater Aquaculture for providing necessary facilities for carrying out this work.

References

1. Ali SR, Ambasankar K, Praveena E, et al. Effect of dietary prebiotic inulin on histology, immuno-hematological and biochemical parameters of Asian sea bass (*Lates calcarifer*). Aquac Res. 2018; 9: 2732-40.
2. Ali SR, Ambasankar K, Praveena E, et al. Effect of dietary fructooligosaccharide supplementation on growth, body composition, hematological and immunological parameters of Asian sea bass (*Lates calcarifer*). Aquac Int. 2017; 25(2): 837-48.
3. Kumar JJ, Satayanarayana Y, Kumar MB, et al. Prebioitcs in Aquaculture. Fishing Chimes. 2011; 31: 53-5.
4. Faggio C, Fazio F, Marafioti S, et al. Oral administration of gum Arabic: Effects on hematological parameters and oxidative stress markers in Mugil cephalus, Iran J Fish Sci. 2015; 14(1): 60-2.
5. Ali SR, Ambasankar K, Nandakumar S, et al. Effect of dietary prebiotic inulin on growth, body composition and gut microbiota of Asian sea bass (*Lates calcarifer*). Anim Feed Sci Technol. 2016; 217:87-94.
6. Akrami R, Razeghi M, Chitsaz M, et al. Effect of dietary mannan oligosaccharide on growth performance, survival, body composition and some hematological parameters of carp juvenile (*Cyprinus carpio*). J of Aquac Feed Sci and Nutr. 2012; 4: 54-60.
7. Lokesh J, Fernandes JMO, Korsnes K, et al. Transcriptional regulation of cytokines in the intestine of Atlantic cod fed yeast derived mannan oligosaccharide or B-glucan and challenged with Vibrio anguillarum. Fish Shellfish Immunol. 2012; 33(3): 626-31.
8. Vellaichamy R, Kaliyan M, Thangappan A. et al. The efficacy of dietary yeast mannan oligosaccharide on growth and survival rate in (Amphiprion ocellaris) fingerlings. Eur J of Biotechnol & Biosci. 2013; 1(2): 12-5.
9. Torrecillas S, Montero D, Izquierdo MS, et al. Health and growth of fish fed oligosaccharides: Potential mode of action. Fish & Shellfish Immunol. 2014; 36: 525-44.
10. Bavington CD, Page CP. Stopping bacterial adhesion: a novel approach to treating infections. Respiration. 2015; 72: 335-44.
11. Ali SR, Ambasankar K, Praveena EP, et al. Effect of dietary prebiotic fructooligosaccharide on histology, digestive enzyme activity, biochemical and immunological parameters of Asian sea bass (*Lates calcarifer*) fingerlings. Indian J Fish. 2020; 67(4): 80-8.
12. Hoseinifar SH, Ahmadi AR, Raeisi M, et al. Comparative study on immunomodulatory and growth enhancing effects of three prebiotics (galactooligosaccharide, fructooligosaccharide and inulin) in common carp (*Cyprinus carpio*). Aquac Res. 2017; 48 (7): 3298–307.
13. AOAC. Official methods of analysis of the association of official analytical chemists, 19th edn. Association of official analytical chemists, Arlington, Virgina, USA. 2012.
14. APHA/AWWA/WPCF. Standard methods for the examination of water and waste water, 20th edn. American Public Health Association, New York, USA. 1998.
15. Muyzer G, Brinkhoff T, Nubel U, et al. Denaturing gradient gel electrophoresis (*DGGE*) in microbial ecology. Microb Ecol.1998; 3: 1-27.
16. Ponnusamy L, Xu N, Stav G, et al. Diversity of bacterial communities in container habitats of mosquitoes. Microb Ecol. 2008. 56: 593-603.
17. Wright ES, Yilmaz LS, Noguera DR, et al. DECIPHER a search-based approach to chimera identification for 16S rRNA sequences. Appl Environ Microbiol. 2011; 78: 717-25.
18. Torrecillas S, Makol A, Santana BT, et al. Reduced gut bacterial translocation in European sea bass (Dicentrarchus labrax) fed mannan oligosaccharides (MOS). Fish Shellfish Immunol. 2011; 30: 674-81.
19. Li WF, Zhang XP, Song WH, et al. Effects of Bacillus preparations on immunity and antioxidant activities in grass carp (*Ctenopharyngodon idellus*). Fish Physiol Biochem. 2012; 38: 1585-92.
20. Gu M, Ma HM, Mai KS, et al. Effects of dietary β-glucan, mannanoligosaccharide and their combinations on the growth performance, immunity and resistance against vibrio splendidus of sea cucumber, (*Aposthichious japonicas*). Fish Shellfish Immunol. 2011; 31: 303-9.
21. Holland MC, Lambris JD. The complement system in teleosts. Fish Shellfish Immunol. 2002; 12: 399-20.
22. Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol. 2002; 30: 620-50.
23. Hadas E, Stankiewicz M. Superoxide dismutase and total antioxidant status of larvae and adults of Trichostrongylus colubriformis, Haemonchus contortus and Ostertagia circumcincta. Parasitol Res. 1998; 84: 646-50.
24. Yoshida T, Kruger R, Inglis V, et al. Augmentation of nonspecific protection in African catfish, Clarias gariepinus (*Burchell*), by the long-term oral-administration of immunostimulants. J Fish Dis. 1995; 18: 195-8.

25. Dimitroglou A, Merrifield DL, Spring P, et al. Effect of mannan oligosaccharide (MOS) supplementation on growth performance feed utilization, intestinal histology and gut microbiota of gilthead sea bream (*Sparus aurata*). Aquaculture. 2010; 300: 182-8.

26. Dimitroglou A, Merrifield DL, Moate R, et al. Dietary mannanoligosaccharide supplementation modulates intestinal microbial ecology and improves gut morphology of rainbow trout (*Oncorhynchus mykiss*). J Anim Sci. 2009; 87: 3226 -34.

***Correspondence to:**

S Syed Raffic Aliab
Department of Zoology
ICAR-Central Institute of Brackish water Aquaculture
Tamil Nadu
India
E-mail: syedraffic699@gmail.com