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Growth Performance and Immune Response of Snakehead, *Channa striata* (Bloch 1793) Fed Soy Diets with Supplementation of Mannan Oligosaccharides

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Abstract

This study evaluated the effectiveness of mannan oligosaccharides (MO) supplementation in fish meal (FM), soybean meal (SBM) and soy protein concentrate (SPC) formulated feeds for snakehead, *Channa striata* (Bloch 1793) in a two-way factorial experiment. Factors were diet (FM, 40% FM replacement by SBM, and 40% FM replacement by SPC) and MO supplementation (0%, 0.2%, or 0.4% MO). Growth was significantly affected ($p<0.05$) by diet and MO supplementation, as well as their interaction. Feed conversion ratio, protein efficiency ratio and survival were significantly affected ($p<0.05$) by diet, but only survival was significantly affected ($p<0.05$) by MO supplementation, and interactions were insignificant ($p>0.05$). Red blood cell counts were not significantly affected ($p>0.05$) by diet, MO supplementation, or the interaction, but white blood cell counts were significantly affected ($p<0.05$) by diet and MO supplementation, not the interaction. Immunoglobulin (Ig) levels were significantly increased ($p<0.05$) by MO supplementation and the MO x diet interaction, but diet did not affect Ig levels ($p>0.05$). Following a 15-d bacterial challenge with *Aeromonas hydrophila*, lysozyme levels were significantly increased ($p<0.05$) by MO supplementation and the MO x diet interaction, but not by the diets themselves. Cumulative mortality did not differ among fish fed different diets ($p>0.05$). Our results suggest that MO supplementation may improve diet performance in snakehead culture, although full-scale commercial trials should be conducted to confirm this.

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Introduction

Snakehead is cultured in the Mekong Delta of Vietnam in a variety of farming systems such as ponds, hapas, cages and lined tanks (Sinh and Chung 2010). In 2010, total production of snakehead in the Mekong Delta was about 40,000 tons (Sinh and Chung 2011). However, most snakehead farmers then used the traditional method, feeding fish meal (FM) and trash fish, incurring high production costs. Snakehead is a carnivorous species with a high protein requirement (Samantary and Mohanty 1997; Be and Hien 2010), so feed costs account for 80% of the total snakehead production cost (Hien and Sinh 2013).

Fish meal has traditionally been the main protein source in formulated feeds for carnivorous fish, but FM production is being reduced and prices have increased, so alternative protein sources for aquatic animals are needed. Among plant protein ingredients, soybean meal (SBM) is considered as the most nutritive. However, the high concentration of anti-nutritional factors, such as trypsin inhibitor, antigens, lectins, saponins and oligosaccharides, can have negative effects on digestibility of nutrients and performance of fish (Dersjant-Li 2002). Many soy products are used in aquafeed, e.g. SBM, soy protein concentrate (SPC), and fermented soybean. Fish meal can be replaced by SBM up to 30% in diets for knife fish *Chitala chitala* (Hamilton 1822) (Dan et al. 2013), but only at 20% inclusion in diets for spotted rose snapper *Lutjanus guttatus* (Steindachner 1869) (Silva-Carrillo et al. 2012). Soy protein concentrate (65- 67% crude protein) has had anti-nutritional factors removed by alcohol extraction (Dersjant-Li 2002).

Fish meal can be replaced by SPC from 40 - 100% in diets for rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) (Médale et al. 1998), juvenile cobia *Rachycentron canadum* (Linnaeus 1766) (Salze et al. 2010), and Atlantic cod *Gadus morhua* Linnaeus 1758 (Walker et al. 2010). Moreover, the supply of soy products is more stable and economical than the supply of FM (Hertrampf and Piedad-Pascual 2000). Since Hien et al. (2015) found that replacing 40% of FM by SBM does not affect the survival and growth rate of *C. striata* (but only if phytase is added to countract the impact of phytate in SBM binding minerals), use of commercial feeds containing SBM in snakehead farming has increased rapidly.Ward et al. (2016) showed that SBM-based diets likely contain immunostimulants for summer flounder *Paralichthys dentatus* (Linnaeus 1766), whereas SPC-based diets do not, suggesting that immunostimulants may be removed in the alcohol extraction process in SPC production. Furthermore, Ward (2014) showed that the immunostimulants were most likely the oligosaccharides stachyose and raffinose. Although we wanted to determine whether those compounds would stimulate immune response in snakehead, purified forms of those substances are prohibitively expensive for commercial usage in snakehead diets. We therefore chose to test a commercial mannan oligosaccharide immunostimulant product instead. Intensive farming of snakehead often suffers from disease, with pathogens such as bacteria (*Aeromonas hydrophila* and *Aeromonas sobria*) and fungi (*Aphanomyces invandans*) causing economic losses.

In intensive systems, the fish are sometimes cultured at stocking densities up to 120-160 fish·m^{-2} (Sinh and Chung 2011) and fish easily get stressed and develop infectious diseases, especially epizootic ulcerative syndrome (EUS) (Miles et al. 2001). Additionally, Duc et al. (2012) demonstrated that parasites, fungi and bacteria infected cultured snakehead in An Giang and Dong Thap provinces, Vietnam. Successful supplementation with immunostimulants in diets for aquatic animals can increase resistance to pathogens. Some immunostimulants derive from a fraction of the cell wall of *Saccharomyces cerevisiae*, which is rich in mannan oligosaccharides (MO) and is effectively used as an immunostimulant in many species. For example, supplementation of 0.2% MO in the diets for *O. mykiss*, led to increased growth and survival (Sara et al. 2011). In tilapia *Oreochromis niloticus* (Linnaeus 1758), adding 0.2% MO significantly improved the feed conversion ratio (FCR), lysozyme, and bactericidal activity compared to control, and reduced feed cost by 20% (Ahmad et al. 2014). Significant to our study, addition of 0.2% MO in a FM-based diet for *C. striata* significantly reduced mortality in a challenge with *A. hydrophila* (Talpur et al. 2014).

The aim of this study was therefore to determine whether MO supplementation in diets containing 40% replacement of FM with SBM or SPC improves the growth and immune response of *C. striata*. The study included both a feeding trial and a bacterial challenge experiment. If MO does improve growth and immune response, then a suitable concentration of MO might be used to improve commercial snakehead farming and increase economic efficiency.

Materials and Methods

A feeding trial, designed as a two-way factorial experiment, was conducted to evaluate survival, growth, feed efficiency, and immune response parameters. The first factor included three feed groups, one using only FM as the protein source and the second and third using SBM and SPC, respectively, to replace 40% of the FM. The second factor was the addition of MO either, 0.0% (control), 0.2%, or 0.4% MO (Bio-Mos®, Alltech, USA, a commercial product that contains 25% glucomannoprotein) to each of the feed treatments. Each combination of diet and MO supplementation consisted of 3 replicates. Experimental diets were all formulated to be 45% protein, 9% lipid, and 4.2 Kcal·g^{-1} energy (Table 1). All ingredients were mixed mechanically with water for 30 min (including MO as appropriate) and the dough was then passed through an extruder to obtain pellets of 2-mm diameter.

Diets were dried in direct sunlight for 6 h, then cooled at room temperature for ½ h, and finally stored in airtight plastic bags in cool temperature until use throughout the experiment (i.e., all diets were made at one time only). Proximate composition of diets (Table 1) was analysed using methods of AOAC (2000). Fingerlings used in the experiment were transferred from a hatchery in An Giang province to Can Tho University, acclimated in a 2,000-L circular tank, and fed the control (FM) diet prior to the experiment. Average initial weight per fish was 7.05 g. At the start of the experiment, fingerlings were randomly distributed into 27 composite tanks (500-L capacity, filled with 300 L of water) at a stocking density of 80 fish·tank^{-1}.

Each tank was provided with continuous aeration and flow-through water supply with 30% water exchange·d^{-1}. Fish were fed twice a day (0900 and 1500) to satiation. The amount of consumed feed and uneaten feed in each tank was recorded daily (uneaten feed was siphoned out 30 min after feeding began, dried and weighed). At the end of the experiment, all fish in each tank were counted and weighed for calculation of growth rate and survival. Any fish mortality was recorded daily and dead fish were removed and weighed immediately. Experimental period was 8 weeks. Temperature ranged from 27.5-30.1 ºC, dissolved oxygen from 5.22-5.42 mg·L^{-1}, pH from 7.53-8.01, NO_2^- from 0.62-0.69 mg·L^{-1} and NH_3<0.1 mg·L^{-1}, so the water quality parameters in all treatments were in a suitable range for the normal growth and development of this species. Initial fish weight (W_i) and final fish weight (W_f) were determined before and after the experiment, respectively. Survival rate (SR, %), weight gain (WG; g), FCR, and protein efficiency ratio (PER) were determined as follows (where t = time in days):

$$SR = [(\text{number of fish at the end of experiment}) \cdot (\text{number of initial fish})^{-1}] \times 100$$

$$WG = (W_f - W_i)$$

$$FCR = \text{amount of consumed feed in dry matter} \cdot \text{weight} \cdot \text{gain}^{-1}$$

$$PER = (W_f - W_i) \cdot \text{protein intake}^{-1}$$

After 8 weeks, three fish from each tank were randomly collected and blood withdrawn for analysis of erythrocytes (RBC), leukocytes (WBC), lysozymes and total immunoglobulin (Ig). The remainder of the fish were then tranferred to the bacterial challenge experiment. Red blood cells were counted by the usual method using the Neubauer chamber and Natt – Herrick solution (Natt and Herrick 1952). White blood cells were stained by Wright & Giemsa solution and counted (Hang et al. 2013). Lysozyme was analysed by the method of Ellis (1990). Total Ig was analysed by the method of Siwicki and Anderson (1993), modified by Milla et al. (2010).

Bacterial challenge

A bacterial challenge was conducted after the growth trial to determine the snakehead's resistance to *A. hydrophila*. Fish from each growth trial treatment were divided into two groups. Thus, the experiment consisted of 18 treatments: 9 treatments (3 from the FM group, 3 from the SBM group, and 3 from the SPC group) were injected IP with 0.2 mL physiological saline (0.85%) and the remaining 9 treatments (3 from the FM group, 3 from the SBM group,and 3 from the SPC group) were injected IP with 2.32×10^5CFU·$fish^{-1}$ of bacterial strain CL1403 *A. hydrophila*.

This strain was isolated from *C. striata*, and pathogenicity was determined based on the lethal dose determined by one of the co-authors of the current paper (Duc et al. 2013). Each treatment contained three replicates. Experimental fish had average weights of 49.9-50.7 g and were randomly assigned to 100-L plastic tanks at a density of 15 fish·$tank^{-1}$. During the 15-d experimental period fish were fed their respective diets and dead fish were recorded daily.

For moribund fish, clinical signs were observed by gross inspection, and the lesions were sampled directly for bacteria. Re-isolation and re-identification of bacteria were carried out according to methods of Barrow and Feltham (1993). Water exchange was 20%·d^{-1}. After 15 d, three fish from each replicate were randomly collected to withdraw blood and analyse lysozyme as described for the feeding trial.

Statistical analysis

Results of the feeding trial were analysed by two-way ANOVA followed by Duncan's multiple range test at significance level of 95%. Results of the bacterial challenge experiment were analysed by one-way ANOVA followed by Tukey's range test at significance level of 95%.

Results

In the feeding trial, both final weight (W_f) and weight gain (W_g) were significantly affected by diet and MO supplementation, as well as the interaction between the two (Table 2). In general (with some exceptions), MO supplementation generally improved growth of the fish (Table 2). FCR, PER and survival of fish in this experiment were significantly affected by diet, but only survival was significantly affected by MO supplementation and in no case were the interactions significant (Table 3). Feed conversion ratio was significantly improved (i.e., lower) when fish were fed the SPC diet compared to the SBM diet, but neither was significantly different from fish fed the FM diet (Table 3). Protein efficiency ratio for fish fed the FM and SBM diets was significantly greater than that for fish fed the SBM diet (Table 3). Survival of fish fed SPC diet was significantly lower than that of fish fed the FM and SBM diets, but supplementation with MO, especially at the level of 0.2%, significantly improved survival (Table 3). Red blood cell counts were not significantly affected by either diet, MO supplementation, or the interaction of the two, but WBC counts were significantly affected by both diet and MO supplementation (although not the interaction) (Table 4). Fish fed the SPC diet had significantly higher WBC counts than fish fed the FM diet, but neither group was significantly different from fish fed the SBM diet (Table 4).

Mannan oligosaccharides supplementation at both 0.2% and 0.4% levels significantly increased WBC counts compared to the unsupplemented diets (Table 4). Immunoglobulin levels were significantly increased by MO supplementation and the interaction of MO and diet, but diet did not affect Ig levels (Table 5). At the end of the feeding trial but prior to the bacterial challenge (i.e., "pre-challenge"), lysozyme levels were significantly affected by diet, MO supplementation and the interaction between the two (Table 5). For each diet, the greater the level of MO supplementation, the greater was the level of lysozymes (Table 5). Reduction of fish production costs per kg fish produced, compared to fish fed the FM unsupplemented diet as the standard, ranged from 8.7-15.1% for the various other diets tested (Table 6).

Table 1. Ingredients and proximate chemical composition of experimental diets primarily made of fish meal (FM). soybean meal (SBM) or soy protein concentrate (SPC) with or without supplementation with mannan oligosaccharides (MO).

Ingredients (%)	**FM**	**FM 0.2MO**	**FM 0.4MO**	**SBM**	**SBM 0.2MO**	**SBM 0.4MO**	**SPC**	**SPC 0.2MO**	**SPC 0.4MO**
Kien Giang fishmeal	60.7	60.7	60.7	35.8	35.8	35.8	36.2	36.2	36.2
Defatted soybean meal	-	-	-	33.4	33.4	33.4	-	-	-
SPC	-	-	-	-	-	-	24.07	24.07	24.07
Cassava	23.8	23.6	23.4	8.26	8.06	7.86	20.68	20.48	20.28
Rice bran	10.0	10.0	10.0	15.0	15.0	15.0	10.0	10.0	10.0
Premix mineral and vitamins	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Oil	2.69	2.69	2.69	3.08	3.08	3.08	3.38	3.38	3.38
Carboxymethyl cellulose	0.82	0.82	0.82	0.40	0.40	0.40	3.40	3.40	3.40
Lysine	-	-	-	0.40	0.40	0.40	0.06	0.06	0.06
Methionine	-	-	-	0.28	0.28	0.28	0.24	0.24	0.24
Fish solution	-	-	-	1.50	1.50	1.50	-	-	-
Phytase	-	-	-	0.02	0.02	0.02	-	-	-
Mannan oligosaccharides	0	0.20	0.40	0	0.20	0.40	0	0.20	0.40
Total	100	100	100	100	100	100	100	100	100
The chemical composition of feed (%)									
Crude protein	44.5	44.3	44.8	45.0	44.3	44.3	45.5	44.7	44.2
Crude lipid	8.56	8.62	8.69	8.91	8.45	8.76	8.76	8.83	8.90
Ash	15.4	15.2	15.3	12.5	12.3	12.5	12.18	12.18	12.17
Fiber	1.55	1.61	1.50	2.15	2.16	2.15	4.79	4.75	4.81
NFE	29.99	30.27	29.71	31.4.8	32.7	32.2.9	28.81	29.58	29.95
Energy ($KJ \cdot g^{-1}$)	19.17	19.20	19.25	19.69	19.58	19.61	19.27	19.25	19.22

Premix mineral and vitamin (unitkg^{-1}): Vitamin A. 2.000.000 IU; Vitamin D. 400.000 IU; Vitamin E. 6g; Vitamin B_1. 800mg; Vitamin B_2. 800mg; Vitamin B_{12}. 2mg; Calcium D. Pantothenate. 2g; Folic acid. 160mg;Vitamin C. 15g; Choline Chloride. 100g; Iron (Fe^{2+}). 1g; Zinc (Zn^{2+}). 3g; Manganese (Mn^{2+}). 2g; Copper (Cu^{2+}). 100mg; Iodine (I^-). 20mg; Cobalt (Co^{2+}). 10mg. Mannan oligosaccharideswere the products of Alltech. USA.Fishmeal was from Kien Giang.SPC was a product of Taiwan. Cassava and rice bran were local products.CMC. methionine and lysine were products of Evonik.

Table 2. Growth performance of *Channa striata* fed for 8 weeks on diets based on fish meal (FM) or with 40% of FM replaced by soybean meal (SBM) or soy protein concentrate (SPC) supplemented (or not) with mannan oligosaccharides (MO). W_i is initial weight, W_f is final weight and WG is weight gain. Values (mean ± SD) in a column followed by the same superscript letter are not significantly different (P>0.05). Bottom panel shows results of two-way ANOVA indicating treatment and interaction effects.

Treatment	**W_i (g)**	**W_f (g)**	**WG (g)**
FM	7.00 ± 0.14^{a}	47.7 ± 0.6^{d}	40.7 ± 0.5^{f}
FM 0.2MO	7.05 ± 0.03^{a}	52.5 ± 1.2^{b}	45.5 ± 1.2^{b}
FM 0.4MO	7.01 ± 0.05^{a}	52.1 ± 0.2^{b}	45.0 ± 0.2^{b}
SBM	7.08 ± 0.11^{a}	50.5 ± 2.1^{bc}	43.4 ± 2.0^{bd}
SBM 0.2MO	7.08 ± 0.10^{a}	57.7 ± 1.7^{a}	50.6 ± 1.6^{a}
SBM 0.4MO	7.05 ± 0.06^{a}	57.4 ± 1.5^{a}	50.3 ± 1.5^{a}
SPC	7.23 ± 0.36^{a}	49.4 ± 0.6^{cd}	42.2 ± 0.4^{cf}
SPC 0.2MO	7.00 ± 0.03^{a}	51.3 ± 1.2^{bc}	44.3 ± 1.3^{bc}
SPC 0.4MO	7.06 ± 0.05^{a}	48.9 ± 1.8^{cd}	41.9 ± 1.8^{df}
P values			
Diets	-	*0.000*	*0.000*
MO	-	*0.000*	*0.000*
*Diets*MO*	-	*0.003*	*0.003*

Table 3. Feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate (SR) of *Channa striata* fed for 8 weeks on diets based on fish meal (FM) or with 40% of FM replaced by soybean meal (SBM) or soy protein concentrate (SPC) supplemented (or not) with mannan oligosaccharides (MO).Values (mean ± SD) in a column followed by the same superscript letter are not significantly different(P>0.05). Bottom panel shows results of two-way ANOVA indicating treatment and interaction effects.

Treatment	**Growth parameters**		
	FCR	**PER**	**SR (%)**
Diet sources			
FM	0.97 ± 0.09^{ab}	2.56 ± 0.28^{a}	76.2 ± 3.3^{a}
SBM	1.05 ± 0.08^{a}	2.20 ± 0.24^{b}	78.1 ± 3.9^{a}
SPC	0.93 ± 0.09^{b}	2.67 ± 0.24^{a}	66.6 ± 5.3^{b}
MO levels (%)			
0	1.03 ± 0.20^{a}	2.35 ± 0.57^{b}	67.9 ± 12.4^{b}
0.20	0.94 ± 0.23^{a}	2.53 ± 0.66^{a}	78.8 ± 0.2^{a}
0.40	0.98 ± 0.35^{a}	2.54 ± 0.52^{a}	74.2 ± 9.9^{ab}
P values			
Diets	*0.037*	*0.004*	*0.000*
MO	*0.167*	*0.260*	*0.000*
*Diets*MO*	*0.843*	*0.800*	*0.217*

Table 4. Total red blood cells (RBC) and white blood cells (WBC) in serum of *Channa striata* fed diets based on fish meal (FM) or with 40% of FM replaced by soybean meal (SBM) or soy protein concentrate (SPC) supplemented (or not) with mannan oligosaccharides (MO). Values (mean ± SD) in a column followed by the same superscript letter are not significantly different (P>0.05). Bottom panel shows results of two-way ANOVA indicating treatment and interaction effects.

Treatment	**Blood parameters**	
	RBC (10^6 cells·mm^{-3})	**WBC (10^3 cells·mm^{-3})**
Diet sources		
FM	2.17±0.18^{a}	60.9±5.9^{b}
SBM	2.18±0.33^{a}	66.3±7.1^{ab}
SPC	2.20±0.17^{a}	74.6±3.4^{a}
MO levels (%)		
0	2.17±0.87^{a}	58.6±2.7^{b}
0.20	2.26±0.66^{a}	71.8±1.8^{a}
0.40	2.14±0.52^{a}	71.4±19.8^{a}
P values		
Diets	*0.973*	*0.001*
MO	*0.567*	*0.000*
*Diets*MO*	*0.273*	*0.081*

Table 5. Immunoglobulin (Ig). pre-challenge lysozyme and post-challenge lysozyme levels of *Channa striata* fed diets based on fish meal (FM) or with 40% of FM replaced by soybean meal (SBM) or soy protein concentrate (SPC) supplemented (or not) with mannan oligosaccharides. Values (mean ± SD) in a column followed by the same superscript letter are not significantly different (P>0.05). Bottom panel shows results of two-way ANOVA indicating treatment and iteraction effects.

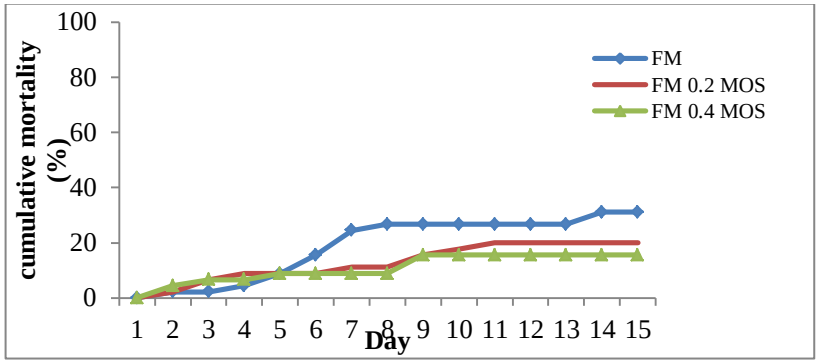
Treatment	**Ig (mg·mL^{-1})**	**Pre-challenge lysozyme (µg·mL^{-1})**	**Post-challenge lysozyme (µg·mL^{-1})**
FM	9.09±0.85^{c}	263±6^{df}	459±3^{df}
FM 0.2MO	10.10±0.57^{bc}	276±13^{d}	503±11^{bc}
FM 0.4MO	12.90±0.83^{a}	346±15^{b}	536±27^{a}
SBM	9.08±0.49^{c}	248±23^{f}	479±6^{cd}
SBM 0.2MO	9.42±0.50^{c}	283±3^{d}	485±7^{c}
SBM 0.4MO	12.00±0.69^{a}	308±14^{c}	529±24^{a}
SPC	8.75±1.36^{c}	271±14^{df}	443±4^{f}
SPC 0.2MO	11.60±0.69^{ab}	323±12^{bc}	524±7^{ab}
SPC 0.4MO	10.20±1.27^{bc}	371±11^{a}	487±3^{c}
P values			
Diets	*0.383*	*0.000*	*0.069*
MO	*0.000*	*0.000*	*0.000*
*Diets*MO*	*0.000*	*0.023*	*0.003*

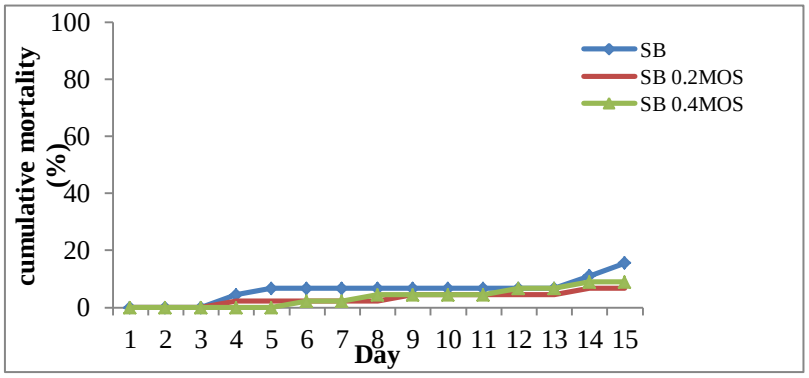
Following the 15-d bacterial challenge with *A. hydrophila*, fish lysozyme levels (i.e., "post-challenge") were significantly increased by MO supplementation and the interaction between MO and diet, but not by the diets themselves (Table 5). Again, the greater the level of MO supplementation, the greater was the lysozyme level (Table 5).

Table 6. Comparision of feed costs (FC), fish production costs (FPC), and reduction in fish production cost compared to control among a diet based completely on fish meal (FM) versus diets in which 40% of FM was replaced by soybean meal (SBM) or soy protein concentrate (SPC). with or without supplementation with mannan oligosaccharides (MO) (based on February 2015 prices).

Treatments	FC (USD·kg^{-1} feed)	FPC (USD·kg^{-1} fish gain)	Reduction in fish production cost (%)
FM	1.063	1.117	0.0
FM 0.2MO	1.072	0.964	13.4
FM 0.4MO	1.085	1.018	8.7
SBM	0.933	1.013	8.9
SBM 0.2MO	0.946	0.955	14.4
SBM 0.4MO	0.955	1.009	9.1
SPC	1.027	0.978	12.3
SPC 0.2MO	1.040	0.946	15.1
SPC 0.4MO	1.049	0.987	11.4

After the 15-d challenge, cumulative mortality was lower for fish given MO supplementation than it was for fish fed the unsupplemented diets (Fig. 1). In general, fish fed the SBM diet, supplemented or not, had lower cumulative mortality than fish fed the FM or SPC diets (Fig. 1).





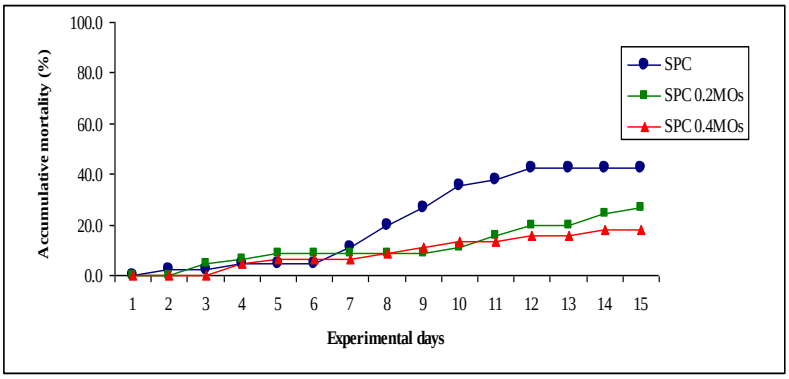


Fig 1. Cumulative mortalities of *Channa striata* during 15-d bacterial challenges with *Aeromonas hydrophila* following a feeding trial in which they had been fed fish meal (FM) diet (top panel), a diet in which soybean meal (SBM) replaced 40% of FM (middle panel) or a diet in which soy protein concentrate (SPC) diet replaced 40% of FM (bottom panel) with or without mannan oligosaccharides(MO) supplementation at 0.2% or 0.4% of the diet (as indicated).

Discussion

The results of the present study confirm that FM can be replaced by SPC at a level of 40% and demonstrate that MO supplementation significantly improves growth and physiological variables, but not survival in a bacterial challenge. Hien et al. (2016) previously showed that SPC can replace 40% of FM in diets for *C. striata*. These results suggest that *C. striata* commercial aquaculture might benefit from the addition of MO to either SBM or SPC diets, although the results should be confirmed in full-scale commercial trials. SPC can replace 75% of FM in diets for *R. canadum* (Salze et al. 2010), 50% of FM in diets for *G. morhua* (Walker et al. 2014) and 40% of FM in diets for Japanese flounder *Paralichthys olivaceus* (Temminck & Schlegel 1846) (Deng et al. 2006), gilthead seabream *Sparus aurata* Linnaeus 1758 (Kissil et al. 2000), and Atlantic salmon *Salmo salar* Linnaeus 1758 (Refstie et al. 2001). SBM can replace up to 30% of FM in diets for carnivorous fish like *C. striata* (Be and Hien, 2010), and *C. chitala* (Hamilton 1822) (Dan et al. 2013). Staykvo el al. (2007) demonstrated that growth and PER of *O. mykiss* given MO was higher, and FCR was lower, than that of fish fed control diets and Sara et al. (2011) also showed that growth increased when feed of rainbow trout was supplemented with 0.2% MO.

FCR and PER of *Diplodus puntazzo* (Walbaum 1792) were not affected when up to 0.8% MO was added to feed in which 40% soy flour replaced by FM (Hernandez et al. 2007). MO also significantly reduced FCR and increased PER in *S. aurata* (Gultepe et al. 2011) and *O. niloticus* (Ahmad et al. 2014). However, in our experiment, MO did not affect FCR or PER, a result also seen in *Dicentrarchus labrax* (Linnaeus 1758) (Torrecillas et al. 2007), *Ictalurus punctatus* (Rafinesque 1818) (Peterson et al. 2010), and *C. striata* (Talpur et al. 2014). Sang et al. (2014) found that shrimp *Penaeus monodon* Fabricius 1798 growth was highest when 1 $g \cdot kg^{-1}$ MO was added to the feed. Increase in number of WBC in fish may serve as protection against pathogenic infection. According to Huong and Tu (2010), leukocytes greatly change under physiological and nutritional conditions of fish and the number of leukocytes can reflect the health status of fish.

Lysozyme plays an important role in the innate immune response and is widely distributed in vertebrates and invertebrates (Magnadóttir et al. 2005). Ahmad et al. (2014) indicated that adding 0.2% MO significantly increased lysozyme and bactericidal activity of *O. niloticus*. Talpur et al. (2014) demonstrated that adding 0.2% MO significantly increased lysozyme of *C. striata*. Jalili et al. (2013) demonstrated no difference in lysozyme and Ig compared to the control when plant-based protein (mainly from SBM) replaced 40, 70 or 100% of FM in diets for *O. mykiss*. Talpur et al. (2014) showed that Bio-Mos at 0.2% in feed for 8 weeks increased Ig and lysozyme in *C. striata.* IgM in serum of experimental fish increased as probiotics (yeast cell wall) were added to the feed of *S. aurata* (Cuesta et al. 2004) and *Salmo coruhensis* Turan, Kottelat & Engin 2010 (Can et al., 2012). Total haemocyte counts were higher in *P. monodon* fed MO in the diet compared to those who were not fed MO (Sang et al. 2014).

Mannan oligosaccharides supplementation appears to improve survival in bacterial challenges for many species. Torrecillas et al. (2007) fed *D. labrax* MO at 0.4% of diet to improve survival and Ahmad et al. (2014) demonstrated that the mortality of *O. niloticus* is 0% when fed diets supplemented with 0.05, 0.1, and 0.2% MO. Addition of 0.2% MO in diet for *C. striata* significantly reduced mortality in a challenge with *A. hydrophila* (Talpur et al. 2014). MO is a prebiotic-type commercial product from Alltech. Mannan oligosaccharides is considered to be a prebiotic because it is not broken down by the host animal`s digestive enzymes, but can be used as a food source for beneficial bacteria that inhabit the natural flora of the digestive tract.

Mannan oligosaccharides does two things in the gut. First, it binds to the attachment sites on certain pathogens and prevents bacteria from attaching to receptors on the host animal`s epithelial gut lining. In doing so, it reduces the pathogens from causing potential damage to the host animal`s digestive tract which can lead to disease. Second, it provides a general stimulation of the immune system by evoking a direct antibody response against invading pathogens. It is interesting that the SBM-fed fish showed (insignificantly) lowest mortality in the bacterial challenge, possibly reflecting the (significant) findings of Ward (2014) that oligosaccharides in SBM likely boost the fish immune system.

Conclusion

Addition of MO to soy-based diets for *C. striata* significantly improves their growth and immune responses. Although survival in the bacterial challenge treatments did not differ significantly, trends were evident in each feed group showing less mortality with MO addition Further research in this area is warranted and addition of MO to *C. striata* diets may improve their performance on commercial farms.

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