

The Effects of Different Salinity and Supplemented Mannan Oligosaccharides (MOS) on Growth of *Litopenaeus vannamei* (Penaeus: Decapoda)

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Abstract: The present study was to test the effects of mannan oligosaccharides (2 and 4 g kg⁻¹) in different salinity (s) levels (20 and 38‰) on growth of white shrimp, *Litopenaeus vannamei*. Research was continued 80 days. For 6 groups and 3 replicates, 18 aquaria were used (80×40×40 cm, 15 *Litopenaeus vannamei*/aquarium, 0.43±0.44 g). Experimental groups were used as M0-s20 (0 g kg⁻¹ MOS-20‰ s), M2-s20 (2 g kg⁻¹ MOS-20‰ s), M4-s20 (4 g kg⁻¹ MOS-20‰ s), M0-s38 (0 g kg⁻¹ MOS-38‰ s), M2-s38 (2 g kg⁻¹ MOS-38‰ s) and M4-s38 (4 g kg⁻¹ MOS-38‰ s). The results of the experiment indicated that the diet supplemented with 0, 2 and 4 g MOS kg⁻¹ in 38‰ salinity (M0-s38, M2-s38, M4-s38) and the control group in 20‰ salinity (M0-s20) enhanced the growth parameters (p<0.05) including the final body weight, live weight gains, daily weight gains and specific growth. At the end of the study the highest live weight gain were detected as 6.65±1.874 g in M4-s38, 6.57±2.134 g in M2-s38, 6.21±2.164 g in M0-s20, 5.88±2.345 g in M0-s38 groups, respectively and the lowest live weight gains were found as 4.66±2.075 g in M2-s20 and 4.74±1.399 g in M4-s20 groups (p<0.05). Respects of the growth and survival rates, the more successful groups were found in 38‰ salinity conditions. Additionally, comparing with the hepatopancreas histology no differences were detected between the groups. In conclusion, MOS supplementation in low salinity level was not promoting the growth parameters in *L. vannamei*.

Key words: Shrimp, *Litopenaeus vannamei*, mannan oligosaccharides, salinity, hepatopancreas histology

INTRODUCTION

It has been suggested that aquaculture could provide new opportunities for food production from the sea and for efficient production systems on land which could expand food production within limited land and water resource constraints. Meeting these needs and achieving these goals will require innovation to refine existing aquaculture techniques and to apply new technologies to responsibly expand production. The scientific and business communities are responding to the challenges and opportunities inherent in the growing aquaculture sector with research efforts generating novel technologies that mirror the diversity of the industry (Browdy *et al.*, 2012). Pacific white shrimp, *Litopenaeus vannamei* is naturally distributed along the Pacific coasts of Central and South America and is extensively farmed for food in many countries including India, Indonesia and Vietnam. Lower production costs and higher yield along with an increasing popularity. The price is the major factor that contributes to increase the production of white shrimp in the world in recent years.

China is the first world producer of white shrimp with a production of 1.32 million tons in 2011 (Zhang *et al.*, 2012; VASEP, 2013). The increasing demand for sustainable aquaculture products has focused attention on the need to improve feeds and feeding to allow increased production and productivity (Browdy *et al.*, 2012).

Prebiotics are known as non-digestible matters that are useful for health-promoting bacteria. Prebiotics are carbohydrates such as monosaccharides, oligosaccharides and polysaccharides. In recent years, prebiotics are widely used for growth enhancer and immunostimulants as alternatives to antibiotics in aquacultures (Zhang *et al.*, 2012). MOS derived from the outer cell wall of yeast (*Saccharomyces cerevisiae*) has been shown to reduce pathogenic bacterial colicization in terrestrial animals and improving gut health and the immune status. Earlier studies demonstrated that dietary MOS could improve growth performance of aquatic animals including penaeid shrimp (Genc *et al.*, 2007a). The MOS supplementations showed significantly enhance growth performance of juvenile white shrimp (*Litopenaeus vannamei*) (Ciger, 2010).

Studies on low salinity subject and its necessity for inland culture of *Litopenaeus vannamei* have been addressed some detailed investigation. Rosas *et al.* (2001) tested 15 and 40‰ salinity levels, Cheng *et al.* (2006), detected dietary Calcium (Ca) and Phosphorus (P) requirements in low-salinity water (2‰) and Li *et al.* (2007) studied at 3, 17 and 32‰ salinity levels and evaluated white shrimps growth performances.

However, the effects of dietary MOS at different salinity levels on *L. vannamei* have not been investigated. The aim of this study was to determine effect of two different dietary Mannan Oligosaccharides (MOS) levels at low and high salinity conditions on growth of juveniles of *Litopenaeus vannamei*.

MATERIALS AND METHODS

The study was carried out at Marine and Freshwater Fish Research Unit (Faculty of Marine Science and Technology, Mustafa Kemal University) in Iskenderun, Turkey. Juvenile white shrimps (*Litopenaeus vannamei*) were acclimatized in a 1000 L fibreglass tank and after an acclimation period of 7 days under laboratory conditions, half of the stock was gradually acclimatized in 20‰ salinity in 3 days. The 15 shrimps were stocked into each of eighteen 120 L aquaria. A total of 270 shrimps (0.43±0.44 g) were used in the experiment.

The aquaria were set in the inside unit with photoperiod (fluorescent light, 12 h) and received continuous aeration. A static system was used and 80% of the water in each aquarium was changed daily. The ranges of physicochemical parameters such as temperature, salinity (YSI Model 30 salinometer; Yellow Springs instrument, Yellow Springs, Ohio, USA) and dissolved oxygen were checked daily throughout the experiment. Water temperature, oxygen and pH were 24±2°C, 6.1-6.4 mg L⁻¹ and 7.2-7.7, respectively. The 20 and 38‰ salinities were managed throughout the experiment.

The phosphorylated Mannan Oligosaccharides (MOS; AQUA-MYCES, Vitomix, Colombia) from the outer portion of the cell wall of the yeast *Saccharomyces cerevisiae* were used as the feed additive (prebiotic). Experimental diets were prepared by supplementing a commercial marine fish diet (Perla MP, Skretting, Italy) by 0, 2 and 4 g MOS per kg diet (g/kg). Dietary MOS doses were determined according to the commercial recommendation of AQUA-MYCES (Genc *et al.*, 2007a, b). Different levels of MOS were blended with mixer for homogenous mix and sufficient water were added to the mix to form a soft dough. The

resultant dough was passed through a mincer with 2 mm diameter die and pellets were dried in room conditions. The diets were given to the shrimps by hand twice a day (at 10:00 and 16:00) for 80 days and all shrimps were weighed individually every 20 days. The amount of diets was adjusted according to the total shrimp biomass calculated for each sampling period (20 days). Each diet (0, 2 and 4 g MOS kg⁻¹ diet) was given to different salinity groups (randomly assigned to triplicate aquaria).

In this study, the experimental groups were designed as M0-s20 (0 g kg⁻¹ MOS-20‰ s), M2-s20 (2 g kg⁻¹ MOS-20‰ s), M4-s20 (4 g kg⁻¹ MOS-20‰ s), M0-s38 (0 g kg⁻¹ MOS-38‰ s), M2-s38 (2 g kg⁻¹ MOS-38‰ s) and M4-s38 (4 g kg⁻¹ MOS-38‰ s).

At the end of the experiment, shrimp of each aquaria were harvested, counted and weighed individually for calculations. The Final Body Weight (FBW), Live Weight Gain (LWG), Daily Weight Gain (DWG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR) and Survival Rate (SR) were calculated according to equations:

$$\text{LWG (g)} = \text{FBW} - \text{Initial Body Weight (IBW)}$$

$$\text{DWG (g day}^{-1}\text{)} = \frac{\text{FBW} - \text{IBW}}{\text{Days}}$$

$$\text{SGR (\% day}^{-1}\text{)} = \frac{\log \text{FBW} - \log \text{IBW}}{\text{Days}} \times 100$$

$$\text{FCR} = \frac{\text{Feed consumption}}{\text{Weight gain}}$$

$$\text{SR (\%)} = \frac{\text{Final number of shrimp} - \text{Initial number of shrimp}}{\text{Initial number of shrimp}} \times 100$$

For proximate composition analysis, five shrimps of each triplicates were pooled and stored at -20°C and analysed according to Association of Analytical Communities (AOAC, 1997) procedures as follows: moisture was determined by oven drying at 105°C for 24 h crude protein (N 6.25) by the Kjeldahl Method and crude ash by combustion in a muffle furnace at 550°C for 16 h. The total lipid concentration was determined by extract with the Chloroform-Methanol Method, described by Bligh and Dyer (1959).

For histological examinations 3 shrimps from each triplicate group were sampled. Hepatopancreas samples were taken from the shrimps and fixed in 4% buffered formalin. After 24 h the fixed tissues were dehydrated with

ethanol (70, 85 and 98%) and clearance with xylene then the samples was vacuum embedded in paraffin and waxed. The rotary microtome studs (3-5 µm; Thermo Shandon, Pittsburgh, PA) were stained with Haematoxylin and Eosin (H&E) and analysed and photographically with an Olympus BX50 microscope (Japan) (Takashima and Hibiya, 1995; Roberts and Smail, 2004).

Data in tables were presented as mean values± Standard Deviation (SD) of 18 aquaria. The final body weight, specific growth rate, live weight gain, feed conversion ratio, survival and proximate composition were subjected to one-way analysis of variance to determine if significant differences occurred among the different salinity and MOS supplementations treatments.

The data were statistically analysed with one-way ANOVA and Duncan's multiple range tests. Effects with a probability of $p < 0.05$ were considered significant. Statistical analyses were performed using SPSS for Windows (SPSS 9.0 Standard Version SPSS Inc., Chicago, Illinois, USA).

RESULTS

The results of the experiment indicated that the diet supplemented with 2 and 4 g MOS kg⁻¹ at 38‰ salinity (M2-s38 and M4-s38) enhanced the growth parameters including the final body weight, live weight gains, daily weight gains and specific growth rate ($p > 0.05$). At the end of the study the highest live weight gain were detected as 6.65±1.874 g in M4-s38, 6.57±2.134 g in M2-s38, 6.21±2.164 g in M0-s20, 5.88±2.345 g in M0-s38 groups, respectively. The lowest live weight gains were found as 4.66±2.075 g in M2-s20 and 4.74±1.399 g in M4-s20 groups and these two groups were found statistically different from other groups ($p < 0.05$).

Respects of the growth and survival rates, the more successful groups were found in 38‰ salinity conditions. Additionally comparing with the hepatopancreas histology no differences were detected between the groups. In conclusion, MOS supplementation in low

salinity level was not promoting the growth parameters in *L. vannamei*. At the end of the study, generally improved growth performance were observed in shrimp fed on diets supplemented with 2 and 4 g MOS kg⁻¹ at 38‰ salinity. The final body weight, live weight gains, daily weight gains and specific growth rate showed better results those groups (M2-s38 and M4-s38) ($p < 0.05$) after 80 days of feeding. The highest live weight gain were detected as 6.65±1.874 g in M4-s38, 6.57±2.134 g in M2-s38, 6.21±2.164 g in M0-s20, 5.88±2.345 g in M0-s38 groups, respectively. The lowest live weight gains were found as 4.66±2.075 g in M2-s20 and 4.74±1.399 g in M4-s20 groups. In addition, feed conversion rates and survival rates of M2-s38 and M4-s38 groups tended to be better than that of the other groups, however without significant differences recorded ($p > 0.05$) (Table 1). The mean live weights of the experimental groups are given in Fig. 1 for the different sampling periods. The body composition of the shrimp were not affected by the different inclusion levels of MOS ($p < 0.05$) (Table 2). In all treatments, the histomorphology of the tubular structures of hepatopancreas tissues were found normal. Different cell types were well recognized. Moreover, all shrimps clinically were healthy. The hepatopancreas proximal studs of *Litopenaeus vannamei* were presented in Fig. 2.

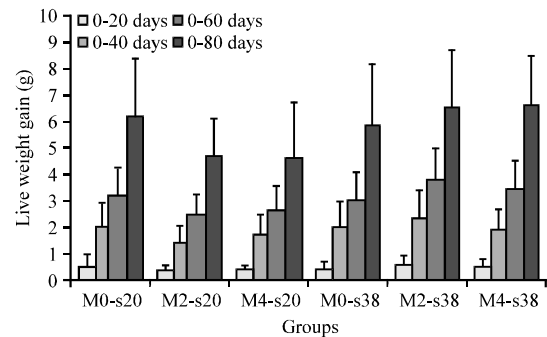


Fig. 1: The mean live weight gains of the experimental groups

Table 1: Growth performances of *Litopenaeus vannamei* fed on diets containing 2 and 4 g kg⁻¹ Mannan Oligosaccharide (MOS) in different salinities (mean±SD)*

MOS (g kg ⁻¹) Groups	Salinity (20‰)			Salinity (38‰)		
	0 M0-s20	2 M2-s20	4 M4-s20	0 M0-s38	2 M2-s38	4 M4-s38
IBW (g)**	0.43±0.006 ^a	0.43±0.011 ⁰	0.43±0.015 ⁰	0.44±0.015 ⁰	0.44±0.006 ⁰	0.44±0.006 ^a
FBW (g)	6.64±2.170 ^b	5.17±1.410 ⁰	5.09±2.090 ⁰	6.32±2.360 ⁰	7.01±2.140 ^b	7.09±1.880 ^b
LWG (g)	6.21±2.160 ^{ab}	4.74±1.400 ⁰	4.66±2.080 ⁰	5.88±2.340 ^{0b}	6.57±2.130 ^b	6.65±1.870 ^b
DWG (g)	0.07±0.024 ^{ab}	0.051±0.008 ^a	0.055±0.017 ^a	0.073±0.008 ^{0b}	0.082±0.007 ^b	0.084±0.011 ^b
SGR (% day ⁻¹)	3.24±0.460 ^{ab}	2.92±0.190 ⁰	2.94±0.370 ^{0b}	3.35±0.170 ^{0b}	3.45±0.100 ^{0b}	3.47±0.160 ^b
FCR	1.90±0.980 ^a	1.96±0.960 ⁰	1.92±0.970 ⁰	1.97±0.980 ⁰	1.87±1.080 ⁰	1.98±1.070 ^a
SR (%)	73.33±6.660 ^a	51.10±9.680 ⁰	57.77±11.110 ⁰	64.44±8.890 ⁰	62.21±18.190 ^a	77.77±4.440 ^a

*Mean values in rows with different superscript are significantly different ($p < 0.05$). **IBW = Initial Body Weight; FBW = Final Body Weight; LWG = Live Weight Gain; DWG = Daily Weight Gain; SGR = Specific Growth Rate; FCR = Feed Conversion Ratio; SR = Survival Rate

Table 2: Proximate composition (dry weight %) of the basal diet and *Litopenaeus vannamei* fed diets containing different levels of Mannan Oligosaccharides (MOS) in different salinities (mean±SD)*

MOS (g kg ⁻¹) Groups	Salinity (20‰)			Salinity (38‰)			Basal diet
	0	2	4	0	2	4	
Dry matter	22.75±0.400	22.16±1.210	23.54±0.60	22.80±1.63	24.77±0.520	23.68±0.470	94
Protein	21.46±0.420	19.56±0.480	20.06±0.35	21.09±0.92	20.65±0.340	20.70±0.620	54
Lipid	1.19±0.002	1.09±0.002	1.22±0.12	1.16±0.08	1.21±0.009	1.11±0.005	18
Crude ash	1.39±0.004	1.30±0.200	1.52±0.31	1.54±0.12	1.49±0.003	1.40±0.004	10

*Mean values (triplicate composite samples of five shrimps) in rows with different superscript are significantly different (p<0.05)

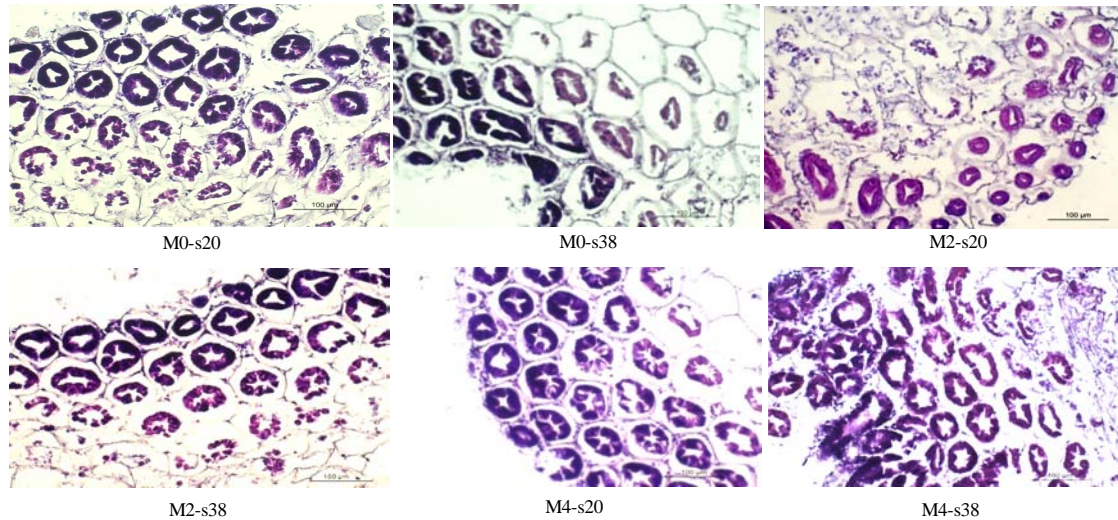


Fig. 2: Hepatopancreas proximal sections of juveniles *Litopenaeus vannamei*: effect of salinity and dietary Mannan Oligosaccharides (MOS) levels (H&E)

DISCUSSION

In spite of the fact that there has been published a very limited research about the MOS supplementation in shrimps particularly *Litopenaeus vannamei*, the successful and encouraging results have been reported. According to Ciger (2010), 3 g kg⁻¹ MOS supplemented diet showed a better improvement in growth performance, furthermore this level of additives in diet revealed positive effects in a very short time period in *L. vannamei* (p<0.05). Also in the present study positive and significantly important results were taken in a very short time period. As it was stated by Ciger (2010)'s study. Zhang *et al.* (2012) reported that Live Weight (LWG) gain and SGR were significantly higher (p<0.05) in *L. vannamei* fed with 2, 4, 6 and 8 g kg⁻¹ MOS supplemented diets than control diet. In their tested groups, they decelerated that the 2 g kg⁻¹ MOS supplemented diet group had a better LWG and SGR result (p<0.05). Genc *et al.* (2007a) stated that in the *Penaeus semisulcatus* which were fed with 3 g kg⁻¹ dietary MOS supplementation, growth performance and FCR had been significantly improved (p<0.05). Earlier studies and reviews have also shown dietary MOS to

improve the growth performance in aquatic animals including in fish and crayfish (Ringo *et al.*, 2010). In the present study the MOS supplementation in 38‰ salinity level resulted a better performance when compared with the 20‰ salinity level (p<0.05). However, between the control groups and the groups with 38‰ salinity no significant differences were detected (p>0.05).

However, for the better growth performance of white shrimps, Rosas *et al.* (2001) suggested that 15‰ salinity than the 40‰ salinity. Cheng *et al.* (2006), claimed that growth rate was decreased tendency in low salinity (2‰) conditions because of lower Calcium (Ca) and Phosphorus (P) levels and Li *et al.* (2007) claimed that at medium salinity (17‰) had best growth performance, when compared with shrimps at either low (3‰) or high salinity levels (32‰). Additionally, Li *et al.* (2007) speculated that based on the literature and the results of their study, suitable salinity for growth of *L. vannamei* would be around 20‰ and other much high or low salinity would adversely affect the shrimp growth. In contrast of Li *et al.* (2007)'s conclusion and Bartlett *et al.* (1990) concluded that growth was not reduced with the range of ambient salinity from 30-45‰ and similar results were

reported by Ponce-Palafox *et al.* (1997). As the white shrimp belongs to the marine species, 38‰ of salinity is its ideal salinity levels, experiments which were done under this salinity level (38‰) with adding 2-4 g kg⁻¹ MOS; revealed a clear improvement of growth rate but on the other hand at the salinity level of 20‰ adding 2-4 g kg⁻¹ MOS were adversely affected in growth parameters (p<0.05) and as a matter of the fact that growth rate improvement of the salinity conditions of 38‰ did not change chemical composition of the carcass quality the protein, lipid, dry matter and crude ash remained the same (p>0.05). This result revealed an absolute achievement remaining the same proximate composition.

As an overview on the present histology results in different salinity levels and MOS supplemented groups when compared with the control groups tubular and lumen epithelial cells were found in normal histomorphology. From that reason, supplementations of dietary MOS at different salinities in hepatopancreatic tissues did not show any adverse effects in the aspect of pathology. Also, in the other studies about the MOS supplementation (Pryor *et al.*, 2003; Sweetman and Davies, 2006; Dimitroglou *et al.*, 2010; Staykov *et al.*, 2007; Yilmaz *et al.*, 2007; Ringo *et al.*, 2010; Genc *et al.*, 2011, 2013) similar results have been reported.

CONCLUSION

The present study showed that supplementation with 2-4 g kg⁻¹ MOS 38‰ salinity levels could significantly improve growth performance and survival rate. From that reason MOS could be used for *L. vannamei* culture. On the other hand; supplementation with any MOS in the 20‰ salinity was found adversely affected in growth performance of *L. vannamei*. Therefore, using any MOS added diets in low salinity (≤20‰) is not recommended.

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