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Short communication

Effects of dietary mannan oligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp, *Litopenaeus vannamei*

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ABSTRACT

An 8-week feeding trial was conducted to investigate the effects of dietary mannan oligosaccharide (MOS) on growth performance, gut morphology, and NH_3 stress tolerance of Pacific white shrimp *Litopenaeus vannamei*. Juvenile Pacific white shrimp (1080 individuals with initial weight of 2.52 ± 0.01 g) were fed either control diet without MOS or one of five dietary MOS (1.0, 2.0, 4.0, 6.0 and 8.0 g kg^{-1}) diets. After the 8-week feeding trial, growth parameters, immune parameters, intestinal microvilli length and resistance against NH_3 stress were assessed. Weight gain (WG) and specific growth rate (SGR) were significantly higher ($P < 0.05$) in shrimp fed 2.0, 4.0, 6.0 and 8.0 g kg^{-1} MOS-supplemented diets than shrimp fed control diet. WG and SGR of shrimp fed 2.0 g kg^{-1} MOS-supplemented diet was the highest ($P < 0.05$) in all experimental groups. Survival rate (SR) of shrimp was generally similar ($P > 0.05$) in all experimental groups. Compared with control group, TEM analysis revealed that 2.0, 4.0, 6.0 and 8.0 g kg^{-1} MOS supplementation could significantly increase ($P < 0.05$) the intestinal microvilli length of shrimp at the ultrastructural level. After NH_3 stress for 24 h, SR of shrimp fed 2.0, 4.0, 6.0 and 8.0 g kg^{-1} MOS-supplemented diets was significantly higher ($P < 0.05$) than that of shrimp fed control diet. Phenoloxidase (PO) activity of 4.0 g kg^{-1} MOS-supplemented group was significantly higher ($P < 0.05$) than that of control group under normal conditions and NH_3 stress. PO activity significantly decreased ($P < 0.05$) under NH_3 stress than under normal conditions. Superoxide dismutase (SOD) activity of 4.0, 6.0 and 8.0 g kg^{-1} MOS-supplemented groups was significantly higher ($P < 0.05$) than that of control group under normal conditions. After NH_3 stress for 24 h, SOD activity of all experimental groups also significantly decreased ($P < 0.05$) compared to normal conditions. These results clearly indicated that dietary MOS could improve growth performance and increase the resistance against NH_3 stress in *L. vannamei*, and the 2.0–4.0 g kg^{-1} MOS supplementation was suitable for *L. vannamei*.

1. Introduction

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. The shrimp culture industry has suffered serious bacterial and viral diseases in the past decades, which has caused the serious economic loss and impeded the sustainable development of the industry throughout the world [1–4]. In recent years, the masses of antibiotics are used to control disease outbreaks in shrimp culture industry, which lead to promote the spread of antibiotic-resistant pathogens in cultured species and in the environment [5–7]. Therefore, it is necessary to search for natural, alternative feed additives to enhance immune capacity and prevent disease outbreaks in aquatic animal. Prebiotics are non-digestible food ingredients that beneficially affect the host by stimulating growth and/or activity of a limited number of beneficial bacteria such as *Lactobacillus* and *Bijidobacter* spp. in the gastrointestinal tract while limiting potentially pathogenic bacteria such as *Salmonella*, *Listeria* and *Escherichia coli* [8]. Recently, prebiotics are widely used as immunostimulants and have shown promise as the environmentally-friendly alternatives to antibiotics in aquaculture [9–13].

As a common prebiotic, mannan oligosaccharide (MOS) is derived from cell wall of *Saccharomyces cerevisiae*, and is widely used in nutrition as natural dietary supplementation to improve gastrointestinal health as well as overall health [14–16]. Previous

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studies demonstrated that dietary MOS could improve growth performance and enhance immune capacity of aquatic animal [17–22]. In fish, the dietary MOS could significantly increase intestinal microvilli length and density of subadult rainbow trout (*Oncorhynchus mykiss*) at ultrastructural level, and thus increase the gut absorptive surface area and enhance the nutrient absorptive ability, and also modulate intestinal microbial communities [14]. The dietary MOS could significantly enhance resistance against salinity stress and increase microvilli length of larval cobia (*Rachycentron canadum*) [20]. The dietary MOS could significantly increase weight gain (WG), decrease feed conversion ratio (FCR) and enhance survival rate (SR) in rainbow trout (*O. mykiss*) [23]. In crustacean, the administration of dietary MOS could significantly enhance WG and specific growth rate (SGR) in juvenile tropic spiny lobster (*Panulirus ornatus*) [13]. The dietary MOS could improve the survival, health status and immunity of marron (*Cherax tenuimanus*) under the bacterial infection and stress conditions caused by air and NH_3 exposures [15]. The 3.0 g MOS kg^{-1} supplementation could significantly improve growth performance and FCR of freshwater crayfish (*Astacus leptodactylus*) [17]. The 0.4% Bio-Mos® supplementation could significantly improve the weight, SGR and average weekly weight gain (AWG), increase total haemocyte count, and also significantly enhance protease activity in hepatopancreas and amylase activity in the guts of freshwater crayfish *Cherax destructor* [18]. The MOS supplementation could significantly improve growth parameters, survival and post-larval condition, and also significantly increase the microvilli length and density in larval and post-larval European lobster (*Homarus gammarus*) at ultrastructural level [22]. However, the effects of dietary MOS on *L. vannamei* have not been investigated. The purpose of this study is to determine whether the administration of dietary MOS in *L. vannamei* could improve growth rates and gut morphology, and/or enhance immunity and resistance against NH_3 stress.

2. Materials and methods

2.1. Test diets

Mannan oligosaccharide (Bio-Mos®, Alltech Inc., USA), which is derived from the cell wall of *S. cerevisiae*, containing minimum of 30% protein, 1.4% crude fat and maximum of 13% crude fiber, was tested at five supplemented levels of 1.0 (MOS1.0), 2.0 (MOS2.0), 4.0 (MOS4.0), 6.0 (MOS6.0) and 8.0 (MOS8.0) g kg^{-1} diet, and the diet without Bio-Mos® (Control) is used as control diet (Table 1). All feed ingredients and supplements were thoroughly mixed with soybean oil, and produced pellets of approximate 1.2 mm in diameter respectively. Subsequently, the pellets were dried in the dark and then stored at −20 °C. All experimental diets contained on average 43.17% crude protein, 6.67% crude fat and 9.62% ash, which were measured with standard methods [24].

2.2. Experimental animal

Juvenile Pacific white shrimp were supplied by Evergreen South Ocean Tech Co. Ltd, Zhanjiang, China. Shrimp were placed in the tanks provided with aerated, recirculating filtered seawater and acclimated to the culture environment for 2 weeks. During the acclimation period, the shrimps were fed the basal diet at the rate of 3% body weight two times daily.

2.3. Culture system

36 indoor cement tanks (1 m length, 1 m wide, 1 m height, 1000 L capacity) were used for the trial. Each tank was provided with a biological filtration recirculating seawater system. During the trial, the pH, temperature, salinity and total ammonium and nitrite of seawater were maintained at 7.8–8.2, 28–29 °C, 29–30‰, 6.5–7.0 mg L^{-1} and 0.06–0.08 mg L^{-1}, respectively.

Table 1
Formulation and chemical proximate composition of the experimental diets.

Ingredients	Dietary treatments					
	Control	MOS1.0	MOS2.0	MOS4.0	MOS6.0	MOS8.0
Ingredients (g kg^{-1} diet)						
Fish meal[a]	300	300	300	300	300	300
Soybean meal[a]	200	200	200	200	200	200
Peanut meal	164	164	164	164	164	164
Wheat flour	220	219	218	216	214	212
Beer yeast	50	50	50	50	50	50
Lecithin	10	10	10	10	10	10
Fish oil	10	10	10	10	10	10
Soybean oil	10	10	10	10	10	10
Phospholipids (purity 97%, pc-60)	10	10	10	10	10	10
Vitamin premix[b]	10	10	10	10	10	10
Mineral premix[c]	10	10	10	10	10	10
Vitamin C	1.0	1.0	1.0	1.0	1.0	1.0
Choline chloride (50%)	5.0	5.0	5.0	5.0	5.0	5.0
Mannan oligosaccharide[d]	0	1.0	2.0	4.0	6.0	8.0
Proximate analysis (g kg^{-1} diet)						
Moisture (%)	92	88	89	92	90	89
Crude protein (%)	421	422	420	419	423	420
Crude lipid (%)	76	78	72	75	77	74
Ash (%)	81	80	83	85	84	83

[a] Fish meal: crude protein 69.4% dry matter, crude lipid 6.2% dry matter; soybean meal: crude protein 49.4% dry matter, crude lipid 2.2% dry matter.
[b] Vitamin mix (g kg^{-1} of diet): vitamin A, 300,000 IU; riboflavin, 500 mg; pyridoxine HCL, 400 mg; cyanocobalamin, 1.2 mg; thiamin, 20 mg; menadione, 40 mg; folic acid, 130 mg; biotin, 10 mg; α-tocopherol, 3000 IU; *myo*-inositol, 8000 mg; calcium pantothenate, 760 mg; nicotinic acid, 200 mg; choline chloride, 8000 mg; vitamin D, 40,000 IU.
[c] Mineral mix (g kg^{-1} of diet): $ZnSO_4 \cdot 7H_2O$, 4 g; $CaCO_3$, 215 g; KCL, 90 g; KI, 0.04 g; NaCl, 40 g; $CuSO_4 \cdot 5H_2O$, 3 g; $CoSO_4 \cdot 7H_2O$, 0.02 g; $FeSO_4 \cdot 7H_2O$, 20 g; $MnSO_4 \cdot H_2O$, 3 g; $MgSO_4 \cdot 7H_2O$, 124 g; $Ca(HPO_4)_2 \cdot 2H_2O$, 500 g.
[d] Mannan oligosaccharide: Bio-Mos; Alltech, USA.

2.4. Feeding trial

After the acclimation period, shrimp were weighed and randomly distributed to 6 experimental groups with similar size (2.52 ± 0.01 g mean initial weight), each of which had 6 replicates. Each replicate had 30 shrimp which was reared with the experimental diet for 56 days. The experimental diets were provided at the rate of 7–8% body weight four times daily by hand to all tanks. Uneaten food and feces were siphoned out, and sufficient seawater was added to maintain 500 L in each tank.

At the end of feeding trial, shrimp of each tank were counted and weighed. SR, final body weight (FBW), WG, SGR and FCR were calculated according to the method of Chou and Shiau [25].

2.5. Intestinal histological examination

Intestinal samples from six shrimp per tank were retained for intestinal histological examination by transmission electron microscopy (TEM). As described previously by Dimitroglou et al. [21], samples were post-fixed in 1% OsO_4 for 1 h and embedded with resin. Resin blocks were sectioned using a diamond knife (~90 nm). Ultrathin sections from each sample were cut and placed on copper grids. Sections were stained with saturated uranyl acetate solution for 30 min, rinsed with distilled water and post-stained with lead citrate for 30 min [26]. Ultrathin sections were then screened with a TEM (FEI Tecnai G2 20, Holland) at 150 kV. All digital images were analyzed with ImageJ 1.36 (National Institutes

Table 2
Growth parameters and survival rate of *Litopenaeus vannamei* fed with different mannan oligosaccharide levels over the 56 days feeding trial.

Parameters	Control	MOS1.0	MOS2.0	MOS4.0	MOS6.0	MOS8.0
[a]IBW, g	2.54 ± 0.02	2.54 ± 0.02	2.51 ± 0.02	2.52 ± 0.02	2.50 ± 0.00	2.50 ± 0.02
[b]FBW, g	9.24 ± 0.27**a**	10.05 ± 0.11**b**	10.75 ± 0.15**b**	10.43 ± 0.22**b**	10.27 ± 0.18**b**	10.23 ± 0.30**b**
[c]WG, %	261.95 ± 10.66**a**	296.69 ± 6.29**ab**	327.15 ± 5.70**c**	308.17 ± 10.62**bc**	307.06 ± 8.70**bc**	308.53 ± 10.53**bc**
[d]SGR, % day^{-1}	2.29 ± 0.05**a**	2.46 ± 0.03**ab**	2.59 ± 0.02**c**	2.51 ± 0.05**bc**	2.51 ± 0.04**bc**	2.51 ± 0.05**bc**
[e]FCR	1.78 ± 0.05**a**	1.55 ± 0.03**bc**	1.44 ± 0.02**c**	1.60 ± 0.07**b**	1.58 ± 0.03**b**	1.61 ± 0.04**b**
[f]SR, %	100.00 ± 0.00	100.00 ± 0.00	99.34 ± 0.66	98.66 ± 1.34	100.00 ± 0.00	98.33 ± 1.14

Values represent means ± SD of six replicates and values in the same row with different letters are significantly different ($P < 0.05$).
[a] IBW (g shrimp^{-1}): initial body wet weight (g).
[b] FBW (g shrimp^{-1}): final body wet weight (g).
[c] WG (%): weight gain = 100 × (final body weight − initial body weight)/initial body weight.
[d] SGR (% day^{-1}): specific growth ratio = 100 × (ln final body weight − ln initial body weight)/total number of experimental days.
[e] FCR: feed conversion ratio = g dry feed consumed/g wet weight gain.
[f] SR: survival rate (%) = 100 × final shrimp number/initial shrimp number.

of Health, USA). TEM images (magnification× 13,500) were analyzed to measure the intestinal microvilli length.

2.6. NH_3 exposure experiment

After the 8-week feeding trial, 36 culture tanks were used for NH_3 exposure trial. Each tank was stocked with 15 shrimp. Shrimp were then exposed to NH_3 by adding NH_4Cl into each tank to obtain NH_4^+ concentration of 14.55 mg L^{-1} which is equal to a concentration of 3.81 mg L^{-1}of NH_3 at a temperature of 28 °C, a salinity of 30‰ and a pH of 8.0 [27]. After NH_3 exposure for 24 h, SR was measured. Haemolymph was sampled at the beginning and at 24 h for NH_3 exposure. Haemolymph of each shrimp was withdrawn from the ventral sinus into a 1 mL sterile syringe and stored at 4 °C for 12 h, and then centrifuged at 10,000× g at 4 °C for 10 min. The supernatant fluid was then transferred into 1 mL centrifuge tube and used to measure phenoloxidase (PO) activity and superoxide dismutase (SOD) activity, respectively.

PO activity was measured by spectrophotometry according to the formation of dopachrome produced from L-dihydroxyphenylalanine (L-DOPA) [28]. 10 μL haemolymph supernatant was evenly mixed with 200 μL phosphate buffer (0.1 mol L^{-1}, pH 6.0) and 10 μL L-DOPA (0.1 mol L^{-1}) in a 96-pore ELISA plate. The optical density at 490 nm was measured using a SpectraMax M5 microplate reader (Molecular Devices, USA).

SOD activity was measured using commercially available kit (Nanjing Jiancheng Bioengineering Institute, China) with the xanthine oxidase method [29]. According to manufactures' instruction, 20 μL haemolymph supernatant was evenly mixed with the buffer and then measured using a SpectraMax M5 spectrophotemeter (Molecular Devices, USA) at 550 nm.

2.7. Data analysis

Data in figures and tables were presented as means ± standard deviation of six tanks. The data were statistically analyzed by statistical software SPSS 17.0 (SPSS, Chicago, IL, USA). One-way ANOVA was used to determine whether significant difference existed among the experimental groups. Duncan's multiple range tests were followed for individual comparisons. Comparisons between haemolymph enzyme activities and NH_3 exposure times were made using Independent-samples *t*-test. A probability (*P*) value of less than 0.05 was considered significant.

3. Results

3.1. Growth performance and survival

The growth performance and survival of shrimp are presented in Table 2. After the 8-week feeding trial, FBW and FCR were significantly higher in the shrimp fed all MOS-supplemented diets than in shrimp fed control diet ($P < 0.05$). WG and SGR were significantly higher ($P < 0.05$) in shrimp fed 2.0 g kg^{-1} MOS-supplemented diet compared to shrimp fed control and 1.0 g kg^{-1} MOS-supplemented diets. Meanwhile, no significant difference ($P > 0.05$) was found in SR among all experimental groups.

3.2. Intestinal histology

An overview of histological analysis is presented in Table 3. TEM analysis demonstrated that 2.0, 4.0, 6.0 and 8.0 g kg^{-1} MOS supplementation could significantly increase ($P < 0.05$) the intestinal microvilli length of shrimp at the ultrastructural level. Moreover, the intestinal microvilli of shrimp fed 2.0 g kg^{-1} MOS-supplemented diet was significantly longer than that of shrimp fed other experimental diets (Fig. 1).

3.3. NH_3 exposure stress

After NH_3 exposure stress for 24 h, SR of all experimental groups is shown in Fig. 2. SR was lowest in the control group, which was also significantly lower ($P < 0.05$) than in 2.0, 4.0, 6.0 and 8.0 g kg^{-1} MOS-supplemented groups. Meanwhile, SR was the highest in 4.0 g kg^{-1} MOS-supplemented group, but SR had no significant difference between 4.0 g kg^{-1} MOS-supplemented group and 2.0, 6.0, 8.0 g kg^{-1} MOS-supplemented groups.

3.4. Immune parameters

The analyses of PO and SOD activities are presented in Table 4. PO activity was significantly higher ($P < 0.05$) in shrimp fed

Table 3
Intestinal morphology of *Litopenaeus vannamei* fed with different mannan oligosaccharide levels over the 56 days feeding trial.

Intestinal morphology	Control	MOS1.0	MOS2.0	MOS4.0	MOS6.0	MOS8.0
Microvilli length, μm	0.92 ± 0.03**a**	1.10 ± 0.07**ab**	2.39 ± 0.14**e**	2.15 ± 0.09**d**	1.66 ± 0.03**c**	1.16 ± 0.06**b**

Values represent means ± SD of six replicates and values in the same row with different letters are significantly different ($P < 0.05$).

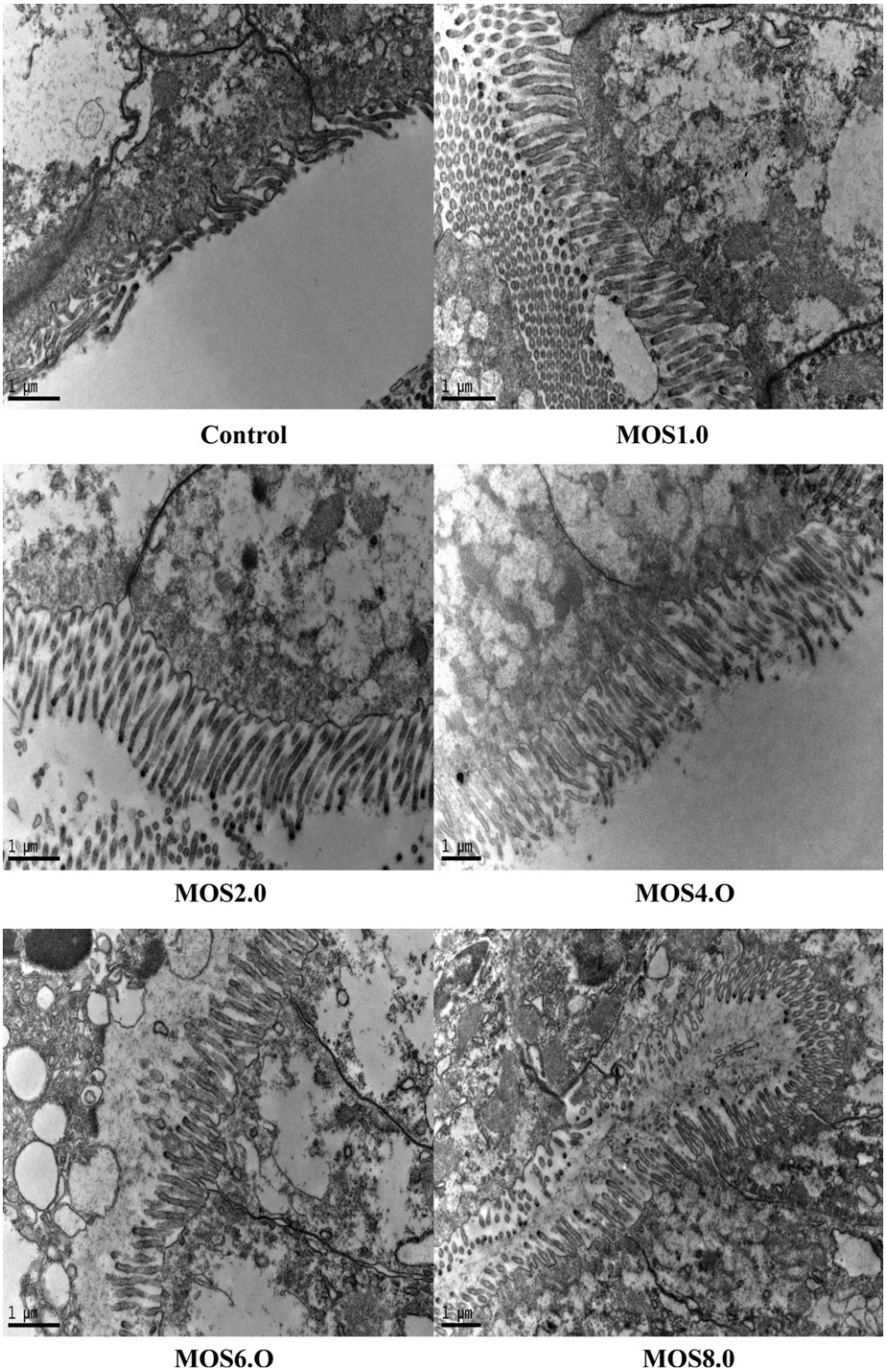

Fig. 1. TEM micrographs from the gut region of *Litopenaeus vannamei* fed control diet, 1.0, 2.0, 4.0, 6.0 and 8.0 g kg^{-1} MOS-supplemented diets after the 56 days feeding trial. Scale bar = 1 μm.

4.0 g kg^{-1} MOS-supplemented diet than in shrimp fed control and 1.0 g kg^{-1} MOS-supplemented diets under normal conditions. After NH_3 exposure for 24 h, PO activity of all experimental groups significantly decreased ($P < 0.05$) under NH_3 stress than under normal conditions. However, PO activity was significantly higher under NH_3 stress in shrimp fed 4.0 g kg^{-1} MOS-supplemented diets than in shrimp fed control and 1.0 g kg^{-1} MOS-supplemented diets.

SOD activity significantly ($P < 0.05$) increased in shrimp fed 4.0, 6.0 and 8.0 g kg^{-1} MOS-supplemented diets than in shrimp fed control diet under normal conditions. After NH_3 stress for 24 h, SOD activity of all experimental groups also significantly decreased ($P < 0.05$) compared to normal conditions, but no significant difference was found among all experimental groups under NH_3 stress.

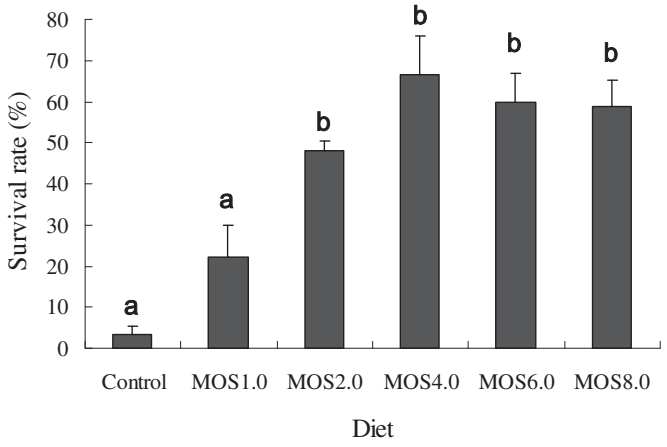

Fig. 2. Survival rate (%) of *Litopenaeus vannamei* fed control diet and MOS diets after NH_3 exposure stress for 24 h.

4. Discussion

The present study demonstrated that dietary MOS could significantly improve the WG and SGR of *L. vannamei*. Previous studies have also shown dietary MOS to improve the growth performance in aquatic animal. The administration of dietary 0.4% MOS could significantly enhance the WG and SGR in tropic juvenile spiny lobster (*P. ornatus*) [13]. The 3.0 g MOS kg^{-1} supplementation could significantly improve growth performance and FCR of freshwater crayfish (*A. leptodactylus*) [17]. The 0.4% Bio-Mos® supplementation could significantly improve the weight, SGR and AWG of freshwater crayfish (*C. destructor*) [18]. The MOS supplementation could significantly improve WG, carapace length, weight to carapace length ratio, SGR and FCR and post-larval condition of European lobster (*H. gammarus*) [22]. The dietary MOS could significantly improve growth performance and the 3.0 g MOS kg^{-1} supplementation had the highest live weight in green tiger prawn (*Penaeus semisulcatus*) [30]. However, other studies demonstrated that dietary MOS could not significantly affect the growth performance of Gulf sturgeon (*Acipenser oxyrinchus* desotoi) [31], hybrid tilapia (*Oreochromis niloticus* × *Oreochromis aureus*) [32], Atlantic salmon (*Salmo salar*) [33], cobia (*R. canadum*) larvae [20] and sea bream (*Sparus aurata*) [21]. These studies suggested that the effects of dietary MOS could vary in different aquatic animals, and thus the effects of dietary MOS should be evaluated before the MOS was used in specific aquatic animals.

Intestinal microvilli provide a vast absorptive surface area, the increase in microvilli length and/or density can increase nutrient absorptive ability [13,34]. In the present study, TEM analysis showed that dietary MOS could significantly increase intestinal microvilli length of *L. vannamei*, which suggested that dietary MOS could improve its nutrient absorptive ability. Previous studies demonstrated that dietary MOS significantly increase the intestinal microvilli length and density in larval and post-larval European lobster (*H. gammarus*) [22]. The dietary MOS could significantly increase the internal perimeter/external perimeter ratio in intestine of juvenile tropical spiny lobsters (*P. ornatus*), which increased gut's absorption surface area [13]. The 0.2% MOS supplementation could increase the intestinal microvilli length of cobia (*R. canadum*) larvae [20]. Similarly, the 0.2% MOS supplementation could also increase the intestinal microvilli density and length of rainbow trout (*O. mykiss*) [21]. These studies suggested that MOS supplementation could produce more intestinal microvilli structures and increase the absorptive surface area in aquatic animals, which should provide the potential to improve nutrient utilization and growth performance. On the contrary, the 0.2% or 0.4% MOS supplementation could not significantly affect the intestinal microvilli length or density of European sea bass (*Dicentrarchus labrax*) [35]. So, the function to intestinal microvilli of MOS supplementation could be different in different aquatic animals, further research need to be done to clarify the mechanism of MOS supplementation to increase intestinal microvilli length and/or density.

The present study showed that 2.0, 4.0, 6.0 and 8.0 g kg^{-1} MOS-supplemented diets could significantly enhance the resistance against NH_3 stress and increase SR of *L. vannamei* under NH_3 stress. Previous study demonstrated that dietary MOS could improve the survival, health status and immunity of marron (*C. tenuimanus*) under the bacterial infection and stress conditions caused by air and NH_3 exposures [15]. The dietary MOS could significantly enhance the resistance against bacterial infection and increase SR of juvenile tropical spiny lobsters (*P. ornatus*) [13]. These results demonstrated that MOS could be used as immunostimulant to enhance resistance against stress and/or bacteria in crustaceans.

PO plays a crucial function in immune defense of invertebrate, which can activate the melanin synthesis pathway, and lead to produce cytotoxic byproducts to kill invading microorganisms [36,37]. PO activity and immunocompetence are positively correlated in numerous invertebrates, and organisms with higher PO activity are less susceptible to infection [38–40]. NH_3 stress has been shown to significantly decrease PO activity of *Macrobrachium rosenbergii* [41]. The present study showed that PO activity was significantly higher in shrimp fed 4.0 g kg^{-1} MOS-supplemented diet under normal conditions. After NH_3 stress for 24 h, PO activity of all experimental groups significantly decreased under NH_3 stress than under normal conditions, but PO activity of shrimp fed 4.0 g kg^{-1} MOS-supplemented diet still was significantly higher than that of shrimp fed control diet. The results suggested that dietary MOS could improve the immune defense capacity and increase the resistance against infection.

Antioxidant responses of living organisms are the important defense mechanisms to resist the exposure of stress environments. SOD is a cytosolic enzyme that is specific for scavenging superoxide radicals, and is involved in protective mechanisms in tissue injury

Table 4
Haemocyte immune parameters of *Litopenaeus vannamei* fed with different mannan oligosaccharide levels over the 0–24 h NH_3 exposure period.

Parameters	Exposure time (h)	Control	MOS1.0	MOS2.0	MOS4.0	MOS6.0	MOS8.0
[a]PO (O.D.490 nm)	0	7.29 ± 0.20A**a**	8.93 ± 1.55A**a**	10.03 ± 0.84A**ab**	13.25 ± 1.15A**b**	9.93 ± 1.34A**ab**	10.27 ± 1.50A**ab**
	24	3.23 ± 0.42B**a**	3.37 ± 0.27B**a**	3.58 ± 1.17B**ab**	5.39 ± 0.48B**b**	5.09 ± 0.59B**ab**	3.77 ± 0.59B**ab**
[b]SOD (unit mg^{-1})	0	13.63 ± 0.33A**a**	14.07 ± 0.83A**a**	15.14 ± 0.42A**ab**	17.37 ± 0.28A**c**	16.89 ± 0.50A**c**	16.30 ± 0.56A**bc**
	24	7.06 ± 0.24Ba	7.49 ± 0.19Ba	7.61 ± 0.49Ba	7.75 ± 0.82Ba	7.54 ± 0.42Ba	7.10 ± 0.41Ba

Values represent means ± SD of six replicates. Significant differences ($P < 0.05$) between the experimental groups and control group are displayed with different non-capitalised letters, while differences between haemocyte immune parameters and exposure times are displayed with capital A and B.
[a] PO (O.D. 490 nm): phenoloxidase activity.
[b] SOD (unit mg^{-1} protein): superoxide dismutase.

following oxidative process and phagocytosis [42]. The 0.1% and 0.2% MOS supplementation could significantly increase SOD activity of sea cucumbers (*Apostichopus japonicus*) [16]. The present study also showed that SOD activity significantly increased in shrimp fed 4.0, 6.0 and 8.0 g kg^{-1} MOS-supplemented diets under normal conditions. The higher SOD activity could be beneficial to increase the resistance against stress environments, which could explain why MOS supplementation increased the SR of *L. vannamei* under NH_3 stress in the present study.

In conclusion, the present study showed that 2.0–4.0 g kg^{-1} MOS supplementation could significantly improve growth performance, increase intestinal microvilli length and enhance the resistance against NH_3 stress, MOS could be used as the immunostimulant in *L. vannamei*.

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