

## Original Research Articles

# Mannan oligosaccharide improves antioxidant capacity, non-specific immunity and protection against *Vibrio* disease and Typhoon stress in *Trachinotus ovatus* juveniles

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This study examined the impact of varying doses of mannan oligosaccharide (MOS) supplementation on antioxidant defense, non-specific immunity, resistance to typhoon stress, and resistance to disease in *Trachinotus ovatus*. Fish groups C, M1, M2, and M3 were fed 1%, 0.3%, 0.6%, and 1% MOS-supplemented feed, respectively. On the 28th and 56th day of the feeding trial, samples from all groups were taken to measure the antioxidant and non-specific immunity parameters and challenged with *Vibrio harvey*. On the 32nd day, a typhoon hit the experimental site, and the survival rates of all groups were calculated. Results showed that on the 28th day, groups M2 and M3 showed considerably higher levels of antioxidant abilities (T-AOC), and groups M1 and M2 showed significantly enhanced non-specific immunity (AKP, LZM, and TP) levels than those of group C ( $p < 0.05$ ). All MOS groups had significantly lower cumulative mortality following the challenge test and typhoon stress than the control group ( $p < 0.05$ ). On the 56th day, groups M2 and M3 showed significantly enhanced antioxidant ability (CAT) compared to the control group. Only one non-specific immunity parameter increased significantly in groups M2 and M3 ( $p < 0.05$ ). After the challenge test, only group M3 exhibited a significantly declined cumulative mortality rate compared to the control groups ( $p < 0.05$ ). In summary, supplementation of MOS at dose 0.6% (M2) for 28 days showed the best overall improving effects on the antioxidant capacity, non-specific immunity, disease resistance, and typhoon stress resistance in *T. ovatus* juveniles.

## INTRODUCTION

Aquaculture is an essential human food source.<sup>1</sup> With the rapid development of intensive aquaculture, unscientific feeding methods have resulted in frequent aquatic animal diseases and economic losses.<sup>2</sup> Common diseases affecting aquatic animals include bacterial virus,<sup>3</sup> and parasitic disease,<sup>4</sup> and *Vibrio harveyi* is a common causative agent of bacterial diseases. Antibiotics and chemicals are frequently employed to treat illnesses, but these approaches lead to issues with drug resistance, environmental contamination, and food safety.<sup>5</sup> Thus, alternatives to antibiotics in aquaculture have received widespread attention, and immune boosters have been used to prevent disease and improve the host's resistance to stress.

Golden pompano (*Trachinotus ovatus*) is a fish belonging to the family *Kamaridae* and the order *Perciformes* and is

widely distributed in tropical and subtropical seas. Due to its fast growth rate, delicious taste, and increasing market demand, it is among the most important species for marine-aquaculture in southern China.<sup>6</sup> The most common culture model for golden pompano was high-density, large scale net cage culture in offshore waters. Therefore, golden pompano farming was vulnerable to extreme weather conditions, such as typhoon and disease outbreaks afterwards. Unfortunately, the literature indicates climate change is likely to cause more frequent and intense extreme weather events and more severe stresses to offshore fish farming.<sup>7</sup> Therefore, it is important to enhance the golden pompano's stress tolerance and overall immunity so that it can survive extreme weather and pathogen infections.

MOS is a glucose protein complex derived from the cell wall of brewer's yeast and is one of the most common prebiotics for aquatic animals.<sup>8</sup> Several studies have shown that

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MOS positively affects aquatic organisms' growth performance, immunity and resistance to stress and diseases.<sup>9</sup> Dietary MOS has been reported to enhance anxiety-like behaviors and stress resistance in zebrafish (*Danio rerio*)<sup>10</sup> and enhance tolerance to air exposure in hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂),<sup>11</sup> improve the resistance of bluntnose seabream (*Megalobrama amblycephala*)<sup>12</sup> against *Vibrio vulnificus* and *Vibrio alginolyticus* and enhance the immune function against *Aeromonas hydrophila* in and grass carp (*Ctenopharyngodon idella*).<sup>13</sup> So far, only one study reported MOS' effect on pompano, enhancing its tolerance to low salinity stress and *Streptococcus iniae*.<sup>14</sup> Being a saltwater fish species cultured in net cages in the subtropical seas, Pompano is more prone to Halophilic pathogens such as *Vibrio* bacteria and natural stresses such as typhoons. Therefore, it is important to evaluate how MOS might protect *T. ovatus* against typhoon and *Vibrio* infection.

In the present study, different supplementary levels of MOS were added to the feed of *T. ovatus*. The effects of MOS on the immune response and stress resistance of *T. ovatus* were investigated by measuring antioxidant capacity, immune capacity, and survival rates after typhoon stress and after *V. harveyi* challenge tests. The findings of this study will provide a theoretical basis for applying MOS in *T. ovatus* aquaculture.

## MATERIALS AND METHODS

### DIET PREPARATION

Commercial feed (52.0% crude protein, 9.0% crude fat, 3.0% crude fiber, 10.0% crude ash, 2.0% calcium, 1.8% total phosphorus, 10.0% moisture, and 3.0% lysine) was used as the basal feed (Guangdong Yuequn Marine Biological Research and Development Co., Ltd., China). Groups with different diets were set up: (1) the control group (C group) was fed a diet containing only basal diet; (2) the M1 group was given a meal that included 3g MOS/kg basal diet; (3) the M2 group was given a meal that included 6g MOS/kg basal diet; and (4) the M3 group was given a meal that included 10g MOS/kg basal diet.<sup>15</sup> To prepare the experimental group rations, 0.3%, 0.6%, and 1% (g/kg) solutions of MOS were used. An equal amount of pure water was sprinkled onto the basic feed for the control group. The same purified water was used to dissolve MOS, which was then uniformly sprayed on the feed. Each mixed meal was allowed to air dry at room temperature. MOS supplement formulations were prepared as needed every 7 days during the feeding trial and stored in plastic bags at 4°C.

### EXPERIMENTAL FISH AND SAMPLE COLLECTION

The feeding trial of *T. ovatus* was carried out on fish rafts in the Lingao Bay, Hainan Province, using small seawater net cages. Before the experiment, the primary feed was administered for one week to allow the fish to adapt to the feed and environment. After 24 hours of fasting, 1200 *T. ovatus* (mean weight 14.41±0.31 g) were randomly assigned to

12 cubic net cages (100 fish/net cage), and three replicates were set up in each group. The fish were fed approximately 3% of their body weight twice a day, at 8:00 and 17:00. The water's temperature varied between 28°C and 30.7°C, with a salinity of 35‰, pH of 7.9 ± 0.2, and less than 0.2 mg/L of ammonia nitrogen.

After 4 weeks and 8 weeks of the feeding experiment, the fish were sampled following 24 hours of fasting. Three sample fish were taken randomly from each net cage (nine fish per group). The sampled fish were first anesthetized with eugenol (100–200 mg/L; Shanghai Medical Instruments Co., Ltd., Shanghai, China).<sup>16</sup> After weighing and disinfecting the fish body surface with alcohol cotton balls, blood was collected via tail vein puncture with a 1ml disposable sterile syringe. The blood was transferred to a centrifuge tube to obtain serum. The blood specimens were left to rest at room temperature for 2 hours, then at 4°C overnight, and finally centrifuged (4°C, 1500rpm, 15 minutes) to separate the serum. The samples were then stored at -80°C for immunological index analysis.<sup>11,15</sup> The head kidney was preserved in Leibovitz's L-15 cell medium (L-15), and respiratory burst was measured. The unsampled fish were weighed and returned to their original cages for subsequent experiments.<sup>15</sup> The research design diagram (Fig. 1) provided illustrations for each of the aforementioned primary sampling processes. All animals were handled strictly in compliance with the rules established by the animal experimentation ethics committee at Hainan University.

### GROWTH PERFORMANCE

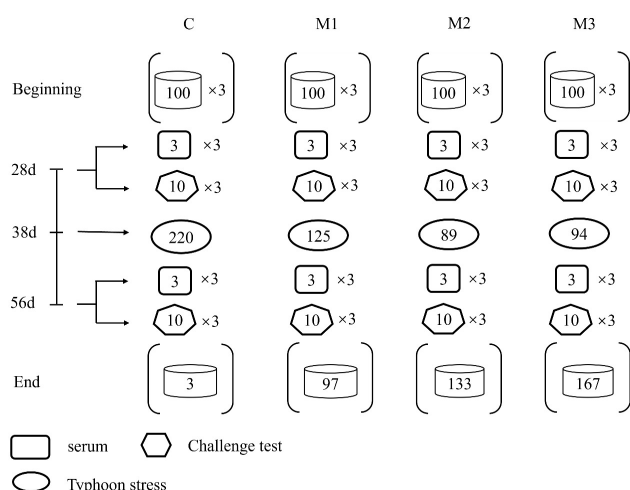
At the beginning and end of the 56-day feeding trial, the total weight of all fish in each cage was measured, and the average weight (the total weight/ the total number of fish) in each cage was used as one replicate for the average weight of each group.<sup>11,15</sup> Specific growth rate (SGR) and weight gain rate were computed.

Weight gain rate (WGR)(%) = 100% × (final average weight – initial average weight) / initial weight

Specific growth rate (SGR)(%) = 100% × [ln(final average weight) – ln(initial average weight)] / feeding days

### THE ACTIVITY OF ANTIOXIDASE AND IMMUNE-RELATED PARAMETERS

A biological replication of the appropriate group was combined with serum samples from three fish in the same experimental group (9 fish/group ÷ 3 fish/pool = 3 pools/group). Serum from fish fed for 4 weeks and 8 weeks was assayed by the instructions of enzyme activity assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China): total antioxidant capacity (T-AOC) (Cat. No. A015), superoxide dismutase (SOD) (Cat. No. A001–3), acid phosphatase activity (ACP)(Cat. No. A060–2), alkaline phosphatase assay kit (AKP) (Cat. No. A059–2), lysozyme (LZM) activity (Cat. No. A050), catalase (CAT) (Cat. No. A007–1–1), albumin (ALB) (Cat. No. A028–2–1), and total protein (TP) (Cat. No. A045–4). Hong et al. followed the approach of measuring macrophages' respiratory burst (RB) using nitroblue tetrazolium (NBT).<sup>17</sup> Using potassium hydroxide



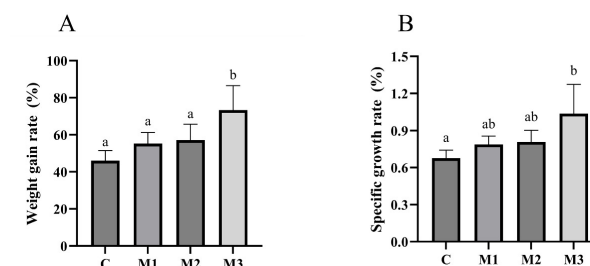
**Figure 1. Diagram of study design.** Samples were collected, respectively, 28 and 56 days after MOS supplementation. Fish in net cages are represented by the number in the barrels. Fish removed for various experimental techniques are represented by the number in the rectangles, hexagons, and ovals. The ovals' numbers indicate how many fish were killed by the storm. The rectangles are sampled fish (tissue and serum); the hexagons are the number of bacterial challenge fish; and the ovals are the number of fish left. C: The diet that was given to the control group consisted solely of basal diet; M1 received diets that included basal diet and 0.3% MOS; M2 received diets that included basal diet and 0.6% MOS; and M3 received diets that included basal diet and 1% MOS.

(KOH)+ dimethyl sulfoxide (DMSO) as a blank, absorbance was measured at a wavelength of 620 nm.

#### CHALLENGE TEST

*V. harveyi* 385 was acquired from Hainan University's (Hainan, China) Collaborative Innovation Center of Marine Science and Technology. The bacteria were grown in Marine Broth 2216E medium and shaken for the entire night at 180 rpm at 30°C. The broth culture was centrifuged at 4°C and 3000rpm/min for 20 minutes. After removing the supernatant, the bacterial pellet was cleaned and added to the phosphate buffer saline (PBS).<sup>18</sup> LD50 ( $1.44 \times 10^7$  CFU/g fish weight) was obtained by preliminary experiments and referring to previously published literature.<sup>19</sup> After 4 and 8 weeks of the feeding experiment, 30 tails in each group were randomly selected for intraperitoneal injection of 100 uL bacterial suspension  $1.44 \times 10^7$  CFU/g fish body weight.<sup>20</sup> The fish were observed for seven days after the bacterial challenge, and mortality was recorded daily. *V. harveyi* 385 was reisolated from dead and clinically infected fish and identified using standard biochemical tests (as described in Ber-gey's Manual of Systematic Bacteriology) and 16S rDNA sequencing methods.<sup>18</sup>

The typhoon stress on the 32<sup>nd</sup> day of the feeding trial, a Typhoon hit the experimental site, which brought tremen-



**Figure 2. Growth performance of *T. ovatus* fed with MOS supplemented diet at the end of 8 weeks of the feeding trial. (A) Weight gain, (B) Specific growth rate. Data is shown as the mean  $\pm$  standard deviation. Additionally, distinct letters above bars denote noteworthy variations ( $p < 0.05$ ).**

dous stress to the experimental fish in the net cages. The cumulative mortality of each group was recorded for 48 hours after the typhoon.

#### STATISTICAL ANALYSIS

The three replicates' mean  $\pm$  standard deviation (SD) values were used to express all data. To examine the data, Duncan's multiple-range tests were employed. Significant statistical differences were indicated by  $p < 0.05$ . SPSS 25.0 was used to analyze the data (SPSS Inc., Chicago, USA).

## RESULTS

#### GROWTH OF *T. OVATUS*

After 8 weeks of MOS feeding, the WGR and SGR were calculated. The results showed that the addition of different concentrations of MOS increased WGR and SGR. Groups M1, M2, and M3's WGR and SGR were significantly higher than group C ( $p < 0.05$ ; Fig. 2).

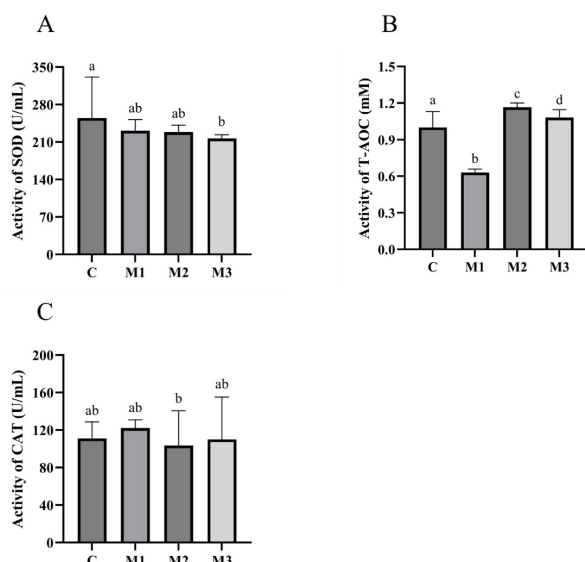
#### ANTIOXIDANT CAPACITY OF *T. OVATUS* AFTER MOS SUPPLEMENTATION

##### 4 WEEKS

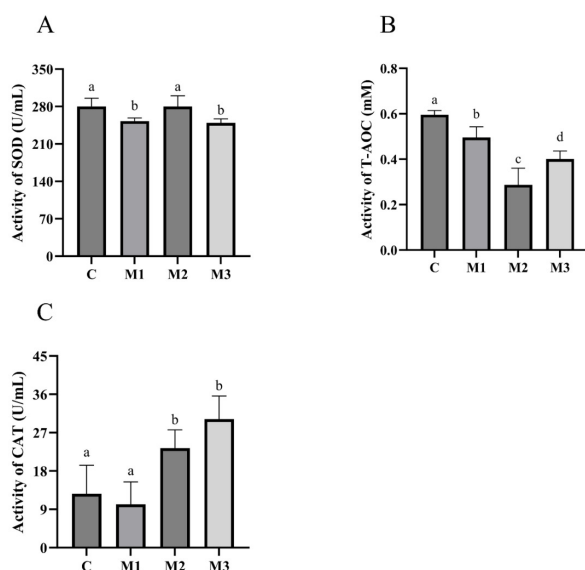
The SOD, T-AOC, and CAT activity levels in the serum were measured, and the results are shown in Figure 3. Compared to group C, group M1 showed significantly reduced T-AOC levels ( $p < 0.05$ ); group M2 showed significantly increased T-AOC levels ( $p < 0.05$ ); and group M3 showed significantly increased T-AOC levels ( $p < 0.05$ ) and significantly reduced SOD levels ( $p < 0.05$ ).

##### 8 WEEKS

The SOD, T-AOC, and CAT activity levels in the serum were measured and shown in Figure 3. Compared to group C, group M1 showed significantly reduced SOD and T-AOC levels ( $p < 0.05$ ); group M2 showed significantly increased CAT levels ( $p < 0.05$ ) and significantly reduced T-AOC levels

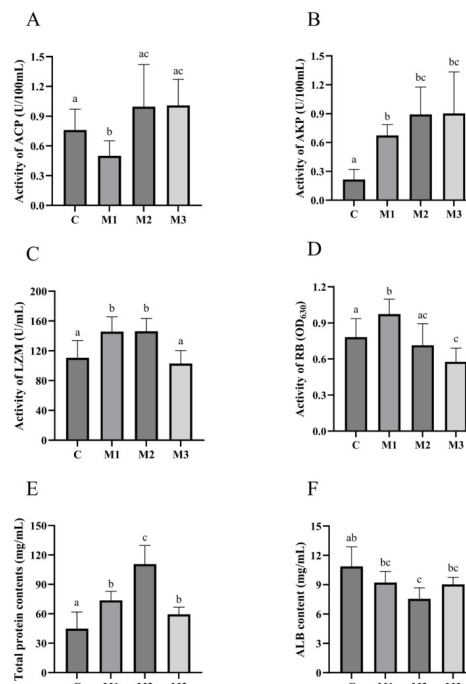


**Figure 3.** Antioxidant capacity of *T. ovatus* after different levels of MOS supplementation for 4 weeks. (A) SOD, (B) T-AOC, and (C) CAT activities of *T. ovatus*. Data is shown as the mean  $\pm$  standard deviation. Additionally, distinct letters above bars denote noteworthy variations ( $p < 0.05$ ).



**Figure 4.** Antioxidant capacity of *T. ovatus* after different levels of MOS supplementation for 8 weeks. (A) SOD, (B) T-AOC, and (C) CAT activities of *T. ovatus*. Data is shown as the mean  $\pm$  standard deviation. Additionally, distinct letters above bars denote noteworthy variations ( $p < 0.05$ ).

( $p < 0.05$ ). Group M3 showed significantly increased CAT level ( $p < 0.05$ ) and significantly reduced SOD and T-AOC levels ( $p < 0.05$ ).



**Figure 5.** Non-specific immunity of *T. ovatus* after different levels of MOS supplementation for 4 weeks. (A) ACP, (B) AKP, (C) LZM, (D) RB, (E) TP, and (F) ALB activities of *T. ovatus*. Data is shown as the mean  $\pm$  standard deviation. Additionally, distinct letters above bars denote noteworthy variations ( $p < 0.05$ ).

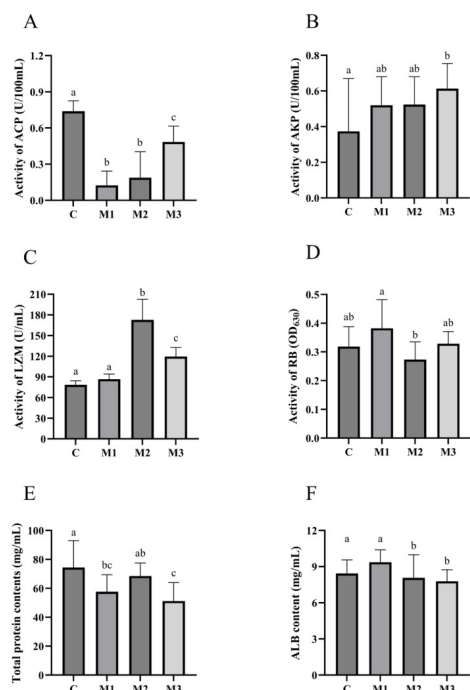
#### NON-SPECIFIC IMMUNITY OF *T. OVATUS* AFTER MOS SUPPLEMENTATION

##### 4 WEEKS

The serum ACP, AKP, LZM, RB, TP, and albumin (ALB) activities were assayed. As shown in Figure 5, compared to group C, group M1 showed 4 significantly up-regulated non-specific immunity (AKP, LZM, RB, and TP) ( $p < 0.05$ ) and one significantly down-regulated non-specific immunity (ACP) ( $p < 0.05$ ). Compared to group C, group M2 showed three significantly up-regulated non-specific immunity (AKP, LZM, and TP) ( $p < 0.05$ ) and one significantly down-regulated non-specific immunity (ALB) ( $p < 0.05$ ). Compared to group C, group M3 showed two significantly up-regulated non-specific immunity (AKP and TP) ( $p < 0.05$ ) and one significantly down-regulated non-specific immunity (RB) ( $p < 0.05$ ).

##### 8 WEEKS

The serum ACP, AKP, LZM, RB, TP, and albumin (ALB) levels were measured. As shown in Figure 6, compared to group C, group M1 showed two significantly down-regulated non-specific immunity (ACP and TP) ( $p < 0.05$ ). Compared to group C, group M2 showed two significantly up-regulated non-specific immunity (LZM and ALB) ( $p < 0.05$ ) and one significantly down-regulated non-specific immunity (ACP) ( $p < 0.05$ ). Compared to group C, group M3 showed two sig-



**Figure 6. Non-specific immunity of *T. ovatus* after different levels of MOS supplementation for 8 weeks.** (A) ACP, (B) AKP, (C) LZM, (D) RB, (E) TP, and (F) ALB activities of *T. ovatus*. Data is shown as the mean  $\pm$  standard deviation. Additionally, distinct letters above bars denote noteworthy variations ( $p < 0.05$ ).

nificantly up-regulated non-specific immunity (AKP and LZM) ( $p < 0.05$ ) and three significantly down-regulated non-specific immunity (ACP, TP, and ALB) ( $p < 0.05$ ).

#### CHALLENGE TEST

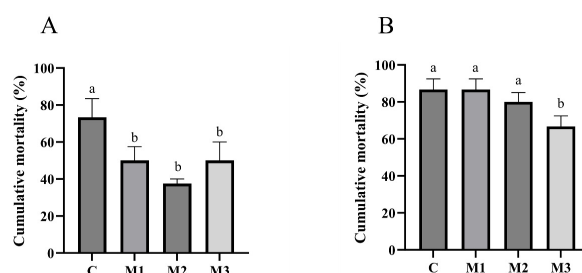
The challenge test results on day 28 revealed that, in comparison to group C, the cumulative mortality rates of all MOS-supplemented groups (M1, M2, and M3) were significantly lower ( $p < 0.05$ ). Only group M3's cumulative mortality rates at day 56 were significantly lower than group C ( $p < 0.05$ ).

#### TYPHOON STRESS

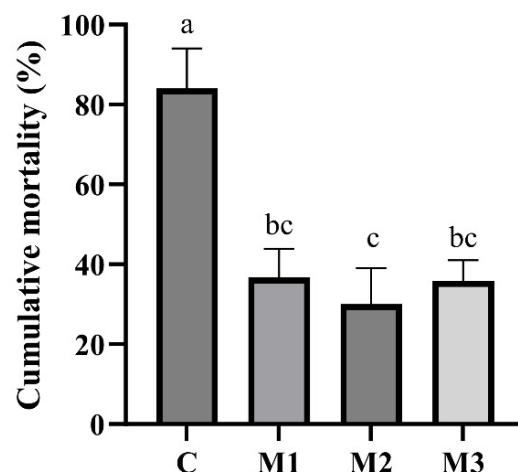
Compared to group C, all three MOS-supplemented groups showed significantly lower cumulative mortality rates ( $p < 0.05$ ).

#### DISCUSSION

Numerous aquatic animals have shown the growth-promoting properties of MOS: Japanese eel (*Anguilla japonica*),<sup>21</sup> and grass carp (*Ctenopharyngodon idellus*)<sup>13</sup> for example. On the contrary, no growth-promoting effects of MOS, regardless of dose levels, were discovered in juvenile blunt snout bream (*Megalobrama amblycephala*).<sup>12</sup> These differences may be due to differences in factors, such as MOS



**Figure 7. The effects of different levels of supplementary MOS on the cumulative mortality of *T. ovatus* after (A) 4 and (B) 8 weeks of attack by *V. harveyi*.** Data is shown as the mean  $\pm$  standard deviation. Additionally, distinct letters above bars denote noteworthy variations ( $p < 0.05$ ).



**Figure 8. The cumulative mortality of *T. ovatus* supplementary with different levels of MOS after typhoon stress.** Data is shown as the mean  $\pm$  standard deviation. Additionally, distinct letters above bars denote noteworthy variations ( $p < 0.05$ ).

chemical structure, feeding time and dosage, fish species, size, age, and culture environment.

The antioxidant response of a biological organism is an essential defense against environmental stress. According to Huang et al. (2015), SOD's primary job is to convert superoxide radicals into molecular oxygen and hydrogen peroxide. In contrast, CAT catalyzes the conversion of hydrogen peroxide into water and oxygen.<sup>22</sup> According to,<sup>23</sup> T-AOC is a direct indicator of fish's total antioxidant capacity. The current findings demonstrated that *T. ovatus*'s antioxidant capacity was raised by adding MOS to fish feed. This aligns with findings from prior research on aquatic creatures like the Nile tilapia (*Oreochromis niloticus*),<sup>24</sup> and grass carp.<sup>13</sup> In the present study, 0.6% and 1% dietary addition of MOS significantly increased the activity of serum T-AOC (after 4 weeks) and CAT (after 8 weeks) in *T. ovatus*. SOD activity exhibited no differences or significant declines



in the three MOS-supplemented groups. This is inconsistent with MOS studies reported with different species: adding 0.4% MOS (4g/kg) to feed significantly increased the T-SOD activity of juvenile *Penaeus vannamei*.<sup>25</sup> A possible reason for the discrepancy might be that MOS stimulates the production of various beneficial microorganisms and substances and an organism's uptake of these substances selectively increases its T-AOC, CAT, or SOD levels.<sup>12</sup>

Non-specific immunity plays an essential role in the disease resistance mechanism of fish. LZM, AKP, ACP, respiratory burst (RB), ALB, and TP are all non-specific immunity indexes that play vital roles in the body's defense. Lysozymes prevent bacterial growth and colonization by causing cell wall disintegration and, ultimately, bacterial death.<sup>26</sup> When the amount of MOS in the diet increased, the LZM activities and RB of *T. ovatus* continually increased. In numerous studies, 14 MOS supplementation (at various doses and for different supplemental times) has demonstrated its LZM-promoting effects.<sup>2,6</sup> Ren et al. (2020) observed a significant enhancement in the serum LZM level of hybrid grouper (*Epinephelus lanceolatus* ♂ × *E. fuscoguttatus* ♀) after a 9-week supplementation of MOS at doses ranging from 0.3% to 0.6%.<sup>15</sup> In a study conducted by Pereira et al. (2018), it was shown that the introduction of MOS for 45 days, at concentrations ranging from 0.1% to 1.5%, resulted in a significant increase in the level of LZM in the serum of juvenile Nile tilapia.<sup>27</sup> Our investigation findings were consistent with results from previous research studies. Significant increases in LZM levels in *T. ovatus* were seen following both 28-day supplementation of MOS at dosages ranging from 0.3% to 0.6% and 56-day treatment of MOS at doses ranging from 0.6% to 1%. Typical hydrolases that detoxify pollutants and prevent toxin invasion are AKP and ACP.<sup>28</sup> The current study's findings show that at 4 weeks, all three MOS-supplemented groups considerably raised *T. ovatus*'s AKP activities; at 8 weeks, only the 1% MOS group significantly raised the blood AKP levels of *T. ovatus*. Interestingly, AKP level changes seemed to be associated with the survival rates of the challenge test: all three groups exhibited enhanced survival rates at 4 weeks; at 8 weeks, only 1% of the MOS group showed significantly enhanced survival rates. The AKP-enhancing effect of MOS is also reported in many other studies on aquatic animals.<sup>28</sup> For instance, Ding et al. (2022) found that supplementing with 0.02% MOS significantly increased the levels of AKP activities in the serum of juvenile blunt snout bream.<sup>12</sup> The ACP-decreasing effect of MOS shown by the present study was not by the ACP-enhancing effect reported in juvenile blunt snout bream and grass carp. Ding et al. reported that Juvenile blunt snout bream (*Megalobrama amblycephala*) serum ACP levels were markedly elevated by an 8-week MOS supplementation at dosages of 0.02-0.04%.<sup>12</sup> According to Lu et al., adding MOS for 60 days at 0.02-0.1% levels greatly raised the serum ACP level of grass carp (*Ctenopharyngodon Idella*).<sup>29</sup> The variation in MOS doses or fish species may cause the disparity in the effects of MOS in fish. As a defense against pathogen invasion, phagocytosis causes phagocytes to create RB; hence, RB activity is a valuable indicator for assessing fish cellular

immunity.<sup>26</sup> The present study showed that after 28-day supplementation of MOS at doses of 0.3%, RB levels were significantly increased in *T. ovatus*. This is in line with MOS studies in sea bass. It was reported that the 45-day addition of MOS at doses of 0.2-0.6% significantly enhanced the RB level of the sea bass (*Dicentrarchus labrax*) serum.<sup>30</sup> The increased levels of TP and ALB can improve protein-binding digestibility and inhibit bacterial toxins.<sup>26</sup> Our study result showed that 28-day supplementation of MOS at the doses of 0.3-1% induced significant increases in TP levels in *T. ovatus*. A similar TP-enhancing effect of short-term MOS supplementation was observed in Nile tilapia, which was supplemented with MOS for 30 days.<sup>26</sup> Our study result showed that 56-day supplementation of MOS at doses of 0.6% significantly increased ALB levels in *T. ovatus*. 8-week supplementation of MOS at doses of 3% also significantly enhanced the ALB level of juvenile great sturgeon (*Huso huso*) serum.<sup>31</sup> Based on the present study's result and those from previous studies, we noticed that the impact of MOS on non-specific immunity parameters of aquatic animals was not always consistent. The divergent findings from different research could be attributed to variations in fish species, MOS dosages, feeding times, temperatures, and other factors.<sup>26,31</sup>

An aquatic animal's resistance to disease can be considered a comprehensive indicator of the organism's immunity. *V. harveyi* is one of the main disease-causing pathogens that affect *T. ovatus*. The present study showed that after 4-5 weeks of MOS supplementation, all tested MOS groups exhibited significantly increased survival rates after the challenge test and typhoon stress resistance; after 8 weeks of MOS supplementation, however, only one of the three MOS groups showed significantly enhanced survival rates after the challenge test. This "shorter is better" effect of MOS was also observed in hybrid grouper and Atlantic salmon. In hybrid grouper (*Epinephelus lanceolatus* ♂ × *E. fuscoguttatus* ♀), 4-week supplementation of MOS (0.2% and 0.3%) induced enhanced disease resistance and multi-stress resistance (ammonia-nitrogen stress and crowding stress);<sup>32</sup> *T. ovatus* fed MOS had a higher tolerance to low salinity stress and *Streptococcus iniae* challenge<sup>14</sup>; yet at 9 weeks, only one of the 4 MOS groups exhibited increased tolerance to bacterial pathogens.<sup>12</sup> Similarly, in Atlantic salmon, increased disease resistance was observed at MOS supplementation of 5 weeks, yet a non-significant protection effect against disease was observed at 10 weeks.<sup>33</sup> On the other hand, however, different MOS effect pattern was also reported. In channel catfish (*Ictalurus punctatus*), for example, the "longer is better" effect of MOS was observed. Although 4 weeks of MOS addition did not induce any protection against *Edwardsiella ictaluri* in Channel catfish, 6 weeks of MOS addition at the same dosage illustrated a significant protection effect against the same pathogen.<sup>34</sup> Therefore, the above study results illustrated that the function pattern of MOS might be closely associated with fish species.

Our study indicates that MOS supplementation in *T. ovatus* diets could improve growth performance, antioxidant capacity (T-AOC, CAT), non-specific immunity (AKP, LZM,

RB, TP, and ALB), and stress resistance (challenge test and typhoon stress) of *T. ovatus*. In addition, compared to long-term (56 days) supplementation, short-term (28 days) supplementation of MOS seemed to have an overall better-enhancing effect on antioxidant capacity, non-specific immunity, and disease resistance of *T. ovatus* fish. In summary, MOS can be an immunostimulant in the *T. ovatus* culture.

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#### AUTHORS' CONTRIBUTION

Data curation: Zihan Chen (Lead). Methodology: Zihan Chen (Equal), Yue Wu (Equal), Xin Chen (Equal). Writing – original draft: Zihan Chen (Equal), Yan Cai (Equal). Investigation: Yan Cai (Equal), Jianlong Li (Equal). Resources:

Yongcai Zhou (Lead). Supervision: Yongcai Zhou (Lead). Conceptualization: Yongcai Zhou (Equal), Shifeng Wang (Equal). Funding acquisition: Zhenjie Cao (Supporting), Shifeng Wang (Lead). Formal Analysis: Jianlong Li (Lead).

#### ETHICAL APPROVAL – IACUC

In this study, the Instructional Animal Care and Use Committee of Hainan University permitted the animal experiments in advance. All possible efforts were made to reduce the suffering of the experimental animals.

#### DECLARATION OF COMPETING INTEREST

The authors state no competing interest in the present study. The common corresponding authors are Shifeng Wang and Yan Cai.

#### DATA AVAILABILITY

Data will be made available on request.

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## REFERENCES

1. Mohapatra S, Chakraborty T, Prusty AK, et al. Use of different microbial probiotics in the diet of rohu, *Labeo rohita* fingerlings: effects on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal microflora. *Aquaculture Nutrition*. 2012;18(1):1-11. doi:[10.1111/j.1365-2095.00866](https://doi.org/10.1111/j.1365-2095.00866)
2. Iwashita MK, Nakandakare P, Terhune IB, et al. Dietary supplementation with *Bacillus subtilis*, *Saccharomyces cerevisiae* and *Aspergillus oryzae* enhance immunity and disease resistance against *Aeromonas hydrophila* and *Streptococcus iniae* infection in juvenile tilapia *Oreochromis niloticus*. *Fish & Shellfish Immunology*. 2015;43(1):60-66. doi:[10.1016/j.fsi.2014.12.008](https://doi.org/10.1016/j.fsi.2014.12.008)
3. Li P, Yu Q, Li F, et al. First identification of the nervous necrosis virus isolated from cultured golden pompano (*Trachinotus ovatus*) in Guangxi, China. *Journal of Fish Diseases*. 2018;41(7):1177-1180. doi:[10.1111/jfb.12805](https://doi.org/10.1111/jfb.12805)
4. Qiao Y, Shao YX, Pengsakul T, et al. Morphological and molecular characterization of *Ceratomyxa batam* n. sp. (Myxozoa: Ceratomyxidae) infecting the gallbladder of the cultured *Trachinotus ovatus* (Perciformes: Carangidae) in Batam Island, Indonesia. *Parasitology Research*. 2019;118(5):1647-1651. doi:[10.1007/s00436-019-06217-w](https://doi.org/10.1007/s00436-019-06217-w)
5. Liu MM, El-Hossary EM, Oelschlaeger TA, et al. Potential of marine natural products against drug-resistant bacterial infections. *The Lancet Infectious Diseases*. 2019;19(7):E237-E245. doi:[10.1016/S1473-3099\(18\)30711-4](https://doi.org/10.1016/S1473-3099(18)30711-4)
6. Zheng PL, Ma ZH, Guo HY, et al. Ontogenetic development of caudal skeletons in *Trachinotus ovatus* larvae. *South China Fisheries Science*. 2014;10(5):45-50. doi:[10.3969/j.issn.2095-0780.2014.05.007](https://doi.org/10.3969/j.issn.2095-0780.2014.05.007)
7. Yu CH, Mu JE, Ding JX, et al. Relationships between typhoon, climate and crime rates in Taiwan. *Nat Hazards*. 2017;89:871-897. doi:[10.1007/s11069-017-2998-9](https://doi.org/10.1007/s11069-017-2998-9)
8. Soleimani N, Hoseinifar SH, Merrifield DL, et al. Dietary supplementation of fructooligosaccharide (FOS) improves the innate immune response, stress resistance, digestive enzyme activities and growth performance of Caspian roach (*Rutilus rutilus*) fry. *Fish & Shellfish Immunology*. 2012;32(2):316-321. doi:[10.1016/j.fsi.2011.11.023](https://doi.org/10.1016/j.fsi.2011.11.023)
9. Zhang ZS, Hu XC, Diao QY, et al. Macrophage migration inhibitory factor (MIF) of golden pompano (*Trachinotus ovatus*) is involved in the antibacterial immune response. *Developmental & Comparative Immunology*. 2022;133:104445. doi:[10.1016/j.dci.2022.104445](https://doi.org/10.1016/j.dci.2022.104445)
10. Forsatkar MN, Nematollahi MA, Rafiee G, et al. Effects of the prebiotic mannan-oligosaccharide on the stress response of feed deprived zebrafish (*Danio rerio*). *Physiology & Behavior*. 2017;180:70-77. doi:[10.1016/j.physbeh.2017.08.010](https://doi.org/10.1016/j.physbeh.2017.08.010)
11. Wang SF, Tian LJ, Wu Y, et al. An accidental discovery of mannan-oligosaccharide's protection effect against air exposure and its potential mechanism in hybrid grouper (*Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀). *Fish Physiology and Biochemistry*. 2022;48(5):1285-1297. doi:[10.1007/s10695-022-01118-9](https://doi.org/10.1007/s10695-022-01118-9)
12. Ding ZJ, Wang X, Liu YL, et al. Dietary Mannan Oligosaccharides Enhance the Non-Specific Immunity, Intestinal Health, and Resistance Capacity of Juvenile Blunt Snout Bream (*Megalobrama amblycephala*) Against *Aeromonas hydrophila*. *Frontiers in Immunology*. 2022;13:863657. doi:[10.3389/fimmu.2022.863657](https://doi.org/10.3389/fimmu.2022.863657)
13. Lu ZH, Jiang WD, Wu P, et al. Mannan oligosaccharides supplementation enhanced head-kidney and spleen immune function in on-growing grass carp (*Ctenopharyngodon idella*). *Fish & Shellfish Immunology*. 2020;106:596-608. doi:[10.1016/j.fsi.2020.08.035](https://doi.org/10.1016/j.fsi.2020.08.035)
14. Do-Huu H, Nguyen HYNH, Vo HT. Effects of Dietary Mannan Oligosaccharides on Growth, Nonspecific Immunity and Tolerance to Salinity Stress and *Streptococcus iniae* Challenge in Golden Pompano, *Trachinotus ovatus*. *Aquaculture Nutrition*. 2023;15:9973909. doi:[10.1155/2023/9973909](https://doi.org/10.1155/2023/9973909)
15. Ren ZL, Wang SF, Cai Y, et al. Effects of dietary mannan oligosaccharide supplementation on growth performance, antioxidant capacity, non-specific immunity and immune-related gene expression of juvenile hybrid grouper (*Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀). *Aquaculture*. 2020;523:735195. doi:[10.1016/j.aquaculture.2020.735195](https://doi.org/10.1016/j.aquaculture.2020.735195)



16. Liu JX, Guo HY, Zhu KC, et al. Effects of exogenous taurine supplementation on the growth, antioxidant capacity, intestine immunity, and resistance against *Streptococcus agalactiae* in juvenile golden pompano (*Trachinotus ovatus*) fed with a low-fishmeal diet. *Frontiers in Immunology*. 2022;13:1036821. doi:[10.3389/fimmu.2022.1036821](https://doi.org/10.3389/fimmu.2022.1036821)
17. Hong XT, Xiang LX, Shao JZ, et al. The immune-stimulating effect of bacterial genomic DNA on the innate immune responses of bivalve mussel, *Hyriopsis cumingii* Lea. *Fish & Shellfish Immunology*. 2006;21:357-364. doi:[10.1016/j.fsi.2005.12.013](https://doi.org/10.1016/j.fsi.2005.12.013)
18. Xu XD, Liu KF, Wang SF, et al. Identification of pathogenicity, investigation of virulent gene distribution and development of a virulent strain-specific detection PCR method for *Vibrio harveyi* isolated from Hainan Province and Guangdong Province, China. *Aquaculture*. 2017;468:226-234. doi:[10.1016/j.aquaculture.2016.10.015](https://doi.org/10.1016/j.aquaculture.2016.10.015)
19. Du HH, Zhou YC, Du XY, et al. Insulin-like growth factor binding protein 5b of *Trachinotus ovatus* and its heparin-binding motif play a critical role in host antibacterial immune responses via NF- $\kappa$ B pathway. *Frontiers in Immunology*. 2023;14(1126843):19. doi:[10.3389/fimmu.2023.1126843](https://doi.org/10.3389/fimmu.2023.1126843). PMID:36865533
20. Cai Y, Wang WF, Guo WL, et al. Transcriptome analysis provides insights into the immune responsive pathways and genes in the head kidney of tiger grouper (*Epinephelus fuscoguttatus*) fed with *Spatholobus suberectus*, *Phellodendron amurense*, or *Eclipta prostrata*. *Fish & Shellfish Immunology*. 2018;73:100-111. doi:[10.1016/j.fsi.2017.12.004](https://doi.org/10.1016/j.fsi.2017.12.004)
21. Lee S, Katya K, Hamidoghli A, et al. Synergistic effects of dietary supplementation of *Bacillus subtilis* WB60 and mannanoligosaccharide (MOS) on growth performance, immunity and disease resistance in Japanese eel, *Anguilla japonica*. *Fish & Shellfish Immunology*. 2018;83:283-291. doi:[10.1016/j.fsi.2018.09.031](https://doi.org/10.1016/j.fsi.2018.09.031)
22. Shao XP, Lin WB, Xu WN, et al. Effects of Dietary Copper Sources and Levels on Performance, Copper Status, Plasma Antioxidant Activities and Relative Copper Bioavailability in *Carassius auratus gibelio*. *Aquaculture*. 2010;308(1-2):60-65. doi:[10.1016/j.aquaculture.2010.07.021](https://doi.org/10.1016/j.aquaculture.2010.07.021)
23. Cheng CH, Yang FF, Ling RZ, et al. Effects of ammonia exposure on apoptosis, oxidative stress and immune response in pufferfish (*Takifugu obscurus*). *Aquatic Toxicology*. 2015;164:61-71. doi:[10.1016/j.aquatox.2015.04.004](https://doi.org/10.1016/j.aquatox.2015.04.004)
24. Dawood MAO, El-Shamaa IS, Abdel-Pazik NI, et al. The effect of mannanoligosaccharide on the growth performance, histopathology, and the expression of immune and antioxidative related genes in Nile tilapia reared under chlorpyrifos ambient toxicity. *Fish & Shellfish Immunology*. 2020;103:421-429. doi:[10.1016/j.fsi.2020.05.061](https://doi.org/10.1016/j.fsi.2020.05.061)
25. Zhang J, Liu YJ, Tian LX, et al. Effects of dietary mannan oligo-saccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific whiteshrimp, *Litopenaeus vannamei*. *Fish & Shellfish Immunology*. 2012;33:1027-1032. doi:[10.1016/j.fsi.2012.05.001](https://doi.org/10.1016/j.fsi.2012.05.001)
26. Hahor W, Thongprajukaew K, Suanyuk N. Effects of dietary supplementation of oligosaccharides on growth performance, gut health and immune response of hybrid catfish (*Pangasianodon gigas* × *Pangasianodon hypophthalmus*). *Aquaculture*. 2019;507:97-107. doi:[10.1016/j.aquaculture.2019.04.010](https://doi.org/10.1016/j.aquaculture.2019.04.010)
27. Peterson BC, Bramble TC, Manning BB. Effects of Bio-MOS on growth and survival of channel catfish challenged with *Edwardsiella ictaluri*. *Journal of the world aquaculture society*. 2010;41:149-155. doi:[10.1111/j.1749-7345.2009.00323](https://doi.org/10.1111/j.1749-7345.2009.00323)
28. Tripathi T, Shahid M, Raza A, et al. Dose-dependent effect of histamine on liver function markers in immunized rabbits. *Experimental and Toxicologic Pathology*. 2012;64:875-881. doi:[10.1016/j.etp.2011.03.010](https://doi.org/10.1016/j.etp.2011.03.010)
29. Lu ZY, Feng L, Jiang WD, et al. An Antioxidant Supplement Function Exploration: Rescue of Intestinal Structure Injury by Mannan Oligosaccharides after *Aeromonas hydrophila* Infection in Grass Carp (*Ctenopharyngodon idella*). *Antioxidants*. 2022;11(5):806. doi:[10.3390/antiox11050806](https://doi.org/10.3390/antiox11050806)
30. Torrecillas S, Makol A, Caballero MJ, et al. Improved feed utilization, intestinal mucus production and immune parameters in sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. *Aquaculture Nutrition*. 2011;17:223-233. doi:[10.1111/j.1365-2095.200900730](https://doi.org/10.1111/j.1365-2095.200900730)
31. Taati R, Bahmani M, Zamini A. Growth performance, carcass composition, and immunophysiological indices in juvenile great sturgeon (*Huso huso*) fed on commercial prebiotic, Immunoster. *Iranian Journal of Fisheries Sciences*. 2011;10(2):324-335.

32. Zhu L, Wang SQ, Cai YS, et al. Effects of Five prebiotics on growth, antioxidant capacity, non-specific immunity, stress resistance, and disease resistance of Juvenile Hybrid Grouper (*Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀). *Animals*. 2023;13(4):754. doi:[10.3390/ani13040754](https://doi.org/10.3390/ani13040754). PMID:36830542

33. Dimitroglou A, Reynold P, Ravny B, et al. The effect of mannan oligosaccharide supplementation on Atlantic salmon smolts (*Salmo salar* L.) fed diets with high levels of plant proteins. *Journal of aquaculture research & development*. Published online 2011:S1-011. doi:[10.41172/2155-9546](https://doi.org/10.41172/2155-9546)

34. Peterson BC, Bramble TC, Manning BB. Effects of Bio-MOS on growth and survival of channel catfish challenged with *Edwardsiella ictaluri*. *Journal of the world aquaculture society*. 2010;41(34):149-155. doi:[10.1111/j.1749-7345.2009.00323](https://doi.org/10.1111/j.1749-7345.2009.00323)