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Research article

Effect of dietary Extracellular polymeric substances from Lactic acid bacteria on disease resistance and immune response in the whiteleg shrimp (*Litopenaeus vannamei*)

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Abstract

Extracellular polymeric substances (EPSs) are known to have prebiotic properties, promoting the growth of beneficial bacteria in the intestinal tracts of humans and animals. To investigate the prebiotic potential of EPSs isolated from lactic acid bacteria (LAB), specifically *Lactiplantibacillus plantarum* and *Bifidobacterium bifidum*, the study examined changes in intestinal LAB and *Vibrio parahaemolyticus* populations, as well as immune responses in the Pacific white shrimp, *Litopenaeus vannamei*. The results showed that dietary supplementation with EPSs increased the population of LAB while reducing the level of *V. parahaemolyticus* in the shrimp gut. Additionally, EPSs were found to enhance the cellular immune responses of the shrimp, including total haemocyte count, respiratory bursts, phenoloxidase activity, and superoxide dismutase activity. Shrimp that were not fed EPSs and later inoculated with V. *parahaemolyticus* registered a mortality rate of 91%. In contrast, shrimp fed with EPSs showed greater resistance to *V. parahaemolyticus*, with mortality rates ranging from 27-30% post-infection. These findings suggest that EPSs produced by LAB could be utilized as a potential prebiotic substitute for antibiotics in shrimp feed to inhibit the growth of *V. parahaemolyticus*, a significant pathogen in aquaculture. The prebiotic and immune-enhancing properties of EPSs demonstrated in this study highlight their potential as a sustainable and eco-friendly approach to disease management in shrimp farming.

Keywords: Lactic acid bacteria, *Litopenaeus vannamei*, Prebiotics, Vibrio parahaemolyticus.

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INTRODUCTION

The Pacific white shrimp, *Litopenaeus vannamei*, accounts for a significant proportion of global crustacean aquaculture (Bardera et al., 2018). However, the aquaculture industry has faced a growing challenge in the form of emerging shrimp diseases, leading to substantial economic losses (Hou et al., 2018). The use of antibiotics in aquaculture has been increasingly restricted due to several concerns. These include the dissemination of antibiotic-resistant bacterial strains, the accumulation of antibiotic residues in the host organisms, the disruption of the intestinal microflora, and the potential reduction in host immunity (Smith et al., 2003; Sapkota et al., 2008; Miller et al., 2018). Concurrently, microbial polysaccharides known as prebiotics (Badel et al., 2011) have gained considerable attention as a natural alternative for the treatment of shrimp infections (Butt et al., 2021). Extracellular polymeric substances (EPSs), which primarily consist of exopolysaccharides, are an important class of these microbial polysaccharides. EPSs have been regarded as a potential natural substitute for chemical medications and antibiotics due to the various health benefits they can provide (Paul et al., 2011).

Prebiotics are non-digestible food ingredients that selectively promote the growth of beneficial bacteria (Hamprakorn et al., 2024), thereby exerting positive effects on host health (Sestito et al., 2020). One of the most prominent effects of prebiotics is their ability to activate the intestinal immune system of various aquatic animals, including the Pacific white shrimp (*L. vannamei*) (Neto and Nunes, 2015; Hu et al., 2019), common carp (*Cyprinus carpio*) (Hoseinifar et al., 2014), Nile tilapia (*Oreochromis niloticus*) (Van Doan et al., 2018), and red drum (*Sciaenops ocellatus*) (Zhou et al., 2010). In addition to their immunomodulatory effects, prebiotics can also be fermented by intestinal bacteria, leading to the production of various metabolites, such as short-chain fatty acids (SCFAs), which possess anti-inflammatory and immunomodulatory properties (Nawaz et al., 2018). Indigestible carbohydrates, including galactooligosaccharides, fructooligosaccharides, inulin, and xylooligosaccharides, are among the most extensively studied and applied prebiotics in aquaculture (Butt et al., 2021). In this context, the EPSs produced by lactic acid bacteria (LAB) have the potential to be employed as prebiotics in shrimp farming (Grosu-Tudor et al., 2013).

LAB are well-known producers of EPSs with a wide range of unique properties, making them valuable for various applications. EPSs derived from LAB exhibit diverse biological and pharmacological activities, rendering them promising as antioxidants, antivirals, anticancer, anti-inflammatory, and immunomodulatory agents in the pharmaceutical and medical industries (Madhuri and Vidya Prabhakar, 2014). In addition to their therapeutic potential, many studies have demonstrated the prebiotic capabilities of LAB-derived EPSs. These polysaccharides have been shown to reduce the formation of pathogenic biofilms and stimulate the growth of probiotic bacteria (Hongpattarakere et al., 2012).

Considering the prebiotic potential of EPSs, the present study aimed to evaluate their influences on the density of LAB and *Vibrio parahaemolyticus* in the digestive system, as well as the immune responses of the Pacific white shrimp (*L. vannamei*). The objective of this work was to elucidate the prebiotic potential of LAB-derived EPSs in aquaculture.

MATERIALS AND METHODS

Preparation of LAB's EPSs

The *Lactobacillus plantarum* VAL6 and *Bifidobacterium bifidum* VAR2 strains used in this study were obtained from the Department of Biotechnology at An Giang University, National University Ho Chi Minh City, Vietnam. The production of EPSs

was carried out using the Man-Rogosa-Sharpe (MRS) broth medium. The cultivation process was conducted in 5-L bioreactors (BIOSTAT, Sartorius Stadium, Germany). Specifically, 5 L of MRS medium was inoculated with 100 mL of an overnight bacterial culture (OD_{595} = 1.5). The pH was maintained at 6.8 by titration with 10 M NaOH, the temperature was kept at 37 °C, and the agitation rate was set to 250 rpm. After 30 hours of cultivation, the EPSs were extracted following the method described by Nguyen et al. (2022). The extraction process involved mixing 100 mL of the supernatant with an equal volume of 2 M NaOH, which was then gently stirred overnight at room temperature. The supernatants were recovered by centrifugation at 8,400 *g* for 20 minutes, and the EPSs were precipitated from the supernatants by adding double the volume of 96% (v/v) cold ethanol. The precipitation was carried out at 4 °C for 48 hours. After a second centrifugation step at 8,400 *g* and 4 °C for 30 minutes, the EPSs were dried at 55 °C until a constant weight was achieved. As a result of this process, two types of EPSs were produced: LP from *L. plantarum* VAL6 and BB from *B. bifidum* VAR2. These EPSs were then subjected to further analysis and evaluation for their potential prebiotic and immunomodulatory effects in the Pacific white shrimp (*L. vannamei*) aquaculture system.

Shrimp culture and feeding trials

All procedures involving laboratory animals in this study were approved by the Animal Ethics Committee of An Giang University, Vietnam National University, Ho Chi Minh City, Vietnam. The experimental shrimp were collected from Kien Giang, Vietnam. The shrimp were acclimated and fed an untreated formulated diet twice daily for 10 days in fiberglass tanks under laboratory conditions (25‰ salinity and 27 ± 1.64 °C). After the acclimation period, the shrimp were weighed (8.5 ± 0.1 g) and randomly assigned in equal numbers to 12 fiberglass tanks (500 L, initial density of 50 animals per tank) in four treatment groups, each with three replicates. The feeding trial groups were as follows: a control group (basal diet), a Glucan-treated group (basal diet supplemented with 1,3-β glucan at 4 g/kg of feed), an LP-treated group (basal diet supplemented with LP EPSs at 4 g/kg of feed), and a BB-treated group (basal diet supplemented with BB EPSs at 4 g/kg of feed). The untreated formulated diet was withheld a few hours before administering the experimental diets containing the EPS supplements. During the experiment, the shrimp were fed the respective diets at 5-6% of their body weight, distributed over five meals per day (7:00, 10:00, 13:00, 17:00, and 21:00). Uneaten feed and feces were siphoned out daily. The water temperature, salinity, ammonia-nitrogen, and pH were maintained at 29 ± 1°C, 25 ± 0.5‰, 0.05 ± 0.005 mg/L, and 8.0 ± 0.2, respectively. The commercial shrimp feed provided by Uni-President Vietnam Co., Ltd. was utilized in the shrimp acclimation and feeding trials. Proximate analysis of the basal diet revealed 40% crude protein, 4% crude lipid, 3% crude fiber, 13% ash, and 11% moisture.

Vibrio parahaemolyticus challenge trials

On the 21st day of the feeding trials, the challenge trials were initiated with three replicates. Specifically, 30 shrimp from each replicate of the previous combined feeding trial were equally divided into groups for pathogen challenge. *V. parahaemolyticus* was provided by Research Institute for Aquaculture No. 2 (Ho Chi Minh City, Vietnam). The day before the challenge assay, *V. parahaemolyticus* was cultured for 24 hours at 35 °C on a shaker in 100 mL of 50% sterilized seawater-supplemented brain heart infusion broth (BHI) in 200-mL conical flasks (Immanuel et al., 2004). For the challenge, *V. parahaemolyticus* infection was administered by adding the bacteria directly to the shrimp tanks to achieve a density of 10^5 CFU/mL. This infection level was determined based on our unpublished data, indicating that exposure to this density of the pathogenic bacterium resulted in moderate mortality in the shrimp. During the challenge trials,

shrimp from each treatment group were collected to assess immunological parameters, as well as to determine the densities of LAB and *V. parahaemolyticus* in the shrimp gut.

Determination of bacteria cell density

The densities of LAB and *V. parahaemolyticus* in the shrimp gut were determined at the beginning and after 3, 6, and 9 days of the challenge trial. MRS agar, supplemented with 5% $CaCO_3$, was utilized to enumerate the LAB. The density of *V. parahaemolyticus* was determined using a specialized medium, Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar. Briefly, the shrimp gut was weighed and placed into an Eppendorf tube, where it was ground to break down the gut cells. A saline solution (0.9%) was then added, and the mixture was vortexed carefully. Serial dilutions were prepared from the sample. From the appropriate dilution, 0.1 mL was pipetted onto the center of the agar plate surface. The sample was then spread evenly over the agar using a sterile glass spreader, while simultaneously rotating the Petri dish. After incubation at 37°C for 24 hours, the bacterial colonies were counted to determine the cell densities. LAB colonies were identified by their ability to solubilize $CaCO_3$, while *V. parahaemolyticus* colonies appeared colorless with a green center. This analysis allowed for the assessment of the changes in LAB and *V. parahaemolyticus* populations within the shrimp gut during the course of the challenge trial.

Immune parameters

Immune measurements were conducted on three shrimp from each sample at the beginning of the challenge trial and after 3, 6, and 9 days, following the method previously described by Chiu et al. (2007). Haemolymph samples (100 µL) were withdrawn from the ventral sinus of each shrimp using a 1-mL sterile syringe containing 0.9 mL of an anticoagulant solution. The anticoagulant solution consisted of 30 mM trisodium citrate, 338 mM sodium chloride, and 10 mM EDTA, adjusted to a pH of 7.55 and an osmolality of 115 mM using glucose. The haemolymph samples were then divided into two parts. A drop of the anticoagulant-haemolymph mixture (100 µL) was placed on a haemocytometer to measure the total haemocyte count (THC) using an inverted phase-contrast microscope. The remainder of the haemolymph mixture was used for subsequent immunological tests, such as the determination of phenoloxidase activity, respiratory burst, and other relevant immune parameters.

The phenoloxidase (PO) activity of the shrimp haemocytes was spectrophotometrically measured by recording the formation of dopachrome produced from L-dihydroxyphenylalanine (Sigma-Aldrich), as described by Poulose (2011). Briefly, 50 µL of haemolymph was taken and mixed with 50 µL of 10% sodium dodecyl sulfate and 1.0 mL of L-dihydroxyphenylalanine (0.19% concentration in Tris-HCl buffer). This mixture was then incubated for 30 minutes at 25 °C. The formation of dopachrome was measured every 30 seconds for 3 minutes in a spectrophotometer at 490 nm. The shrimp's PO activity was expressed as the amount of dopachrome formed in 50 µL of haemolymph.

The respiratory burst activity of the shrimp haemocytes was determined by measuring the reduction of nitroblue tetrazolium (NBT) (Sigma-Aldrich) to formazan, as an indicator of superoxide anion production, as described previously by Bell and Smith (1993). Briefly, the assay involved the addition of haemolymph (10 µL) to a reaction mixture containing nitroblue tetrazolium. The formation of formazan, resulting from the reduction of nitroblue tetrazolium by the superoxide anions, was measured using a microplate reader at an optical density of 630 nm. The respiratory burst activity was expressed as the amount of NBT-reduction per 10 µL of haemolymph.

The superoxide dismutase (SOD) activity of the shrimp haemocytes was determined according to the method described by Beauchamp and Fridovich

(1971) using nitroblue tetrazolium in the presence of riboflavin. Briefly, a reaction mixture was prepared containing 0.1 mM EDTA, 13 mM methionine, 0.75 mM NBT, and 20 mM riboflavin in 50 mM phosphate buffer at pH 7.8. An aliquot of haemolymph (0-100 µL) was then added to 2 mL of this reaction mixture. The samples were placed under fluorescent light for 2 minutes or until the optical density at 560 nm reached a value of 0.2-0.25 in the control tubes without haemolymph. The specific SOD activity was expressed as SOD units per milligram of protein. The total protein content of the haemolymph samples was quantified using the Bradford (1976) method, with bovine serum albumin as the standard.

Growth parameters assay

After 21 days of the feeding test, *L. vannamei* was weighed to calculate the specific growth rate (SGR) and evaluated on their survival. The SGR and survival of different treatments were calculated according to the following formula (Li et al., 2018):

Specific growth rate (SGR %) = 100 × (Final bodyweight - Initial bodyweight) / Experiment period

Survival (%) = 100 × N_f /N_i;

in which N_f is number the of live shrimp at the end of the feeding trial and N_i is the initial stocking number of shrimp in the feeding trial

Statistical analysis

The significance of difference was evaluated by the analysis of variance (ANOVA) followed by Duncan's test with statistical software SPSS 17.0 and data are expressed the mean value of three replicates ± SD. The level of statistical significance was set at $p < 0.05$.

RESULTS

Effect of EPSs on the growth

The use of prebiotics has been considered as a viable alternative approach in aquaculture practices to improve growth performance and disease resistance of cultured species. In the present study, the effects of EPSs produced by LAB on the growth and survival of the Pacific white shrimp (*L. vannamei*) were investigated. After a 21-day feeding trial, the results showed that the dietary supplementation of EPSs from LAB sources could significantly enhance the growth of *L. vannamei*. The SGR values of shrimp fed diets supplemented with LP, BB, and Glucan EPSs ranged from 1.8 to 2% per day, which were significantly higher ($p < 0.05$) than the control group (1.2% per day). Similarly, the survival rates of shrimp fed the EPS-supplemented diets were considerably higher compared to the control diet (Table 1).

Table 1 Effects of diets containing EPSs on the SGR and survival of *L. vannamei* after 21 days of feeding

EPSs	SGR (%/day) *	Survival (%)*
LP	2.04 ± 0.08[a]	91.63 ± 2.54[a]
BB	1.85 ± 0.02[c]	92.27 ± 1.96[a]
Glucan	1.94 ± 0.03[b]	91.20 ± 3.46[a]
Control	1.20 ± 0.03[d]	87.03 ± 0.93[b]

*All data are represented as mean ± SD. Different superscript letters in the same column indicate a significant difference ($p < 0.05$, Duncan's test).

Density of LAB and V. parahaemolyticus in the shrimp gut

Prebiotics can stimulate the growth of beneficial microbial populations, such as LAB (Yahfoufi et al., 2018). Meanwhile, probiotics derived from LAB can modulate the intestinal microbiota by maintaining a balanced gut flora and suppressing the growth of pathogenic bacteria in the host (Maldonado Galdeano et al., 2019). To elucidate these correlations, the present study determined the population changes of LAB and the pathogenic bacterium *V. parahaemolyticus* after infection in the shrimp gut. The effects of EPSs on the density of LAB and *V. parahaemolyticus* in the shrimp intestine were shown in Figure 1 and 2, respectively. After the 21-day feeding trials (at time T0), the LAB density in the groups treated with LP, BB, and Glucan EPSs was significantly higher ($p < 0.05$) compared to the control group (Figure 1). These results demonstrated that the diet supplemented with EPSs stimulated the growth of LAB in the shrimp gut. Following the *V. parahaemolyticus* challenge, the intestinal LAB density of the shrimp fed the diets containing LP, BB, and Glucan EPSs steadily increased from day 3 to day 9. However, the LAB density remained relatively unchanged in the control group (Figure 1).

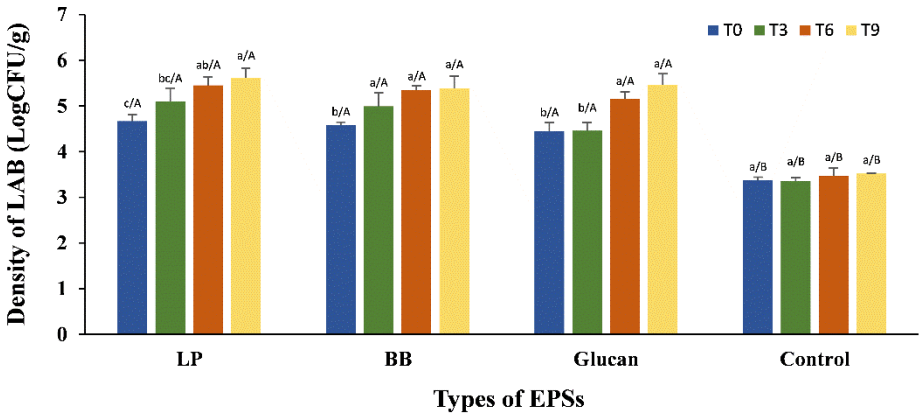

Figure 1 Effect of dietary on the density of LAB in the shrimp gut (T0: before the challenge; T3, T6, and T9 were 3, 6 and 9 days after the challenge, respectively). Different superscript uppercase letters indicate statistically significant differences between treatments at the same sample-collection time and different superscript lowercase letters indicate significant differences throughout time for each separate treatment by Duncan' test ($p < 0.05$)

Regarding the population dynamics of *V. parahaemolyticus*, an opposite trend was observed compared to the LAB density in the shrimp gut (Figure 2). The density of V. parahaemolyticus was significantly lower in the LP, BB, and Glucan EPS groups compared to the control group. Furthermore, the population of *V. parahaemolyticus* gradually decreased over time in the shrimp fed the EPS-

containing diets, while the *V. parahaemolyticus* density gradually increased in the control group (Figure 2). Interestingly, at the initial time point (T0), a certain amount of V. parahaemolyticus was already present in the shrimp gut. This can be explained by the fact that Vibrio species are naturally part of the intestinal microbiota of shrimp and can act as opportunistic pathogens under certain environmental or physiological conditions (Garibay-Valdez et al., 2020).

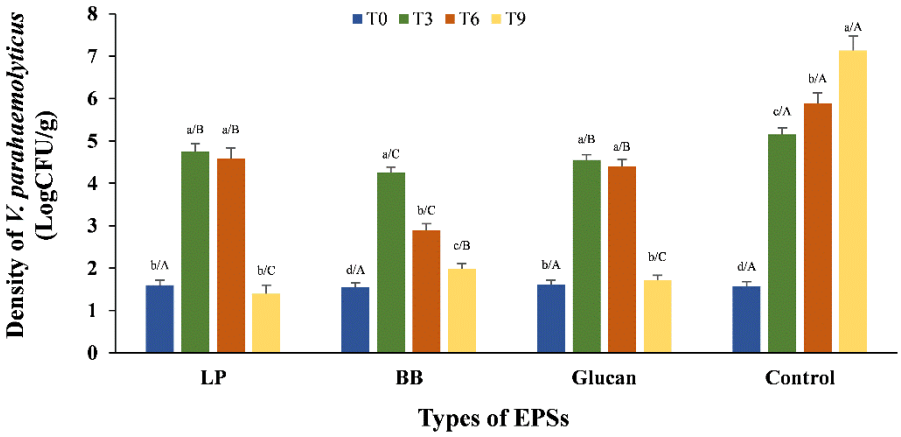

Figure 2 Effect of dietary on the density of *V. parahaemolyticus* in the shrimp gut (T0: before the challenge; T3, T6, and T9 were 3, 6 and 9 days after the challenge, respectively). Different superscript uppercase letters indicate statistically significant differences between treatments at the same sample-collection time and different superscript lowercase letters indicate significant differences throughout time for each separate treatment by Duncan' test ($p < 0.05$)

Immune responses

The results demonstrated that the diet supplemented with EPSs produced by LAB could stimulate the immune responses in the Pacific white shrimp, *L. vannamei.* The effects of dietary EPSs on the total THC, respiratory burst activity, PO activity, and SOD activity in *L. vannamei* are shown in Figs. 3-6, respectively. The THC level in shrimp fed the diets containing LP, BB, and Glucan EPSs increased considerably after 3 days of the challenge ($p < 0.05$), then gradually decreased on days 6 and 9. After 3 days of the challenge, the THC in the shrimp fed the BB diet peaked at 1,366 × 10^3 cells/mL. At the same time, the highest THC in the shrimp fed the LP and Glucan diets was around 650 × 10^3 cells/mL. These values were significantly higher compared to the control (Figure 3).

The PO activity on days 3 and 6 in the EPS groups was significantly lower than that in the control. However, after 9 days of the challenge, the PO activity was significantly higher in the EPS-treated groups compared to the control. Remarkably, the PO activity in the shrimp fed the EPS-containing diets increased sharply on day 9, whereas the PO activity in the control group increased only after 3 days and remained relatively unchanged on the following days (Figure 4).

Similar to PO activity, the respiratory burst activity gradually increased from day 3 to day 6 and peaked on day 9 after the challenge. Furthermore, after 3 days of the challenge, the respiratory burst activity of the shrimp fed the diets containing LP, BB, and Glucan EPSs was higher than that of the shrimp fed the control diet (Figure 5).

In the EPS treatments, while PO activity and respiratory burst activity were highest on day 9 after the challenge, SOD activity was highest on day 6 and then decreased on day 9. The SOD activity of the control was considerably lower than

that of the EPS treatments and remained relatively unchanged during the experimental period (Figure 6).

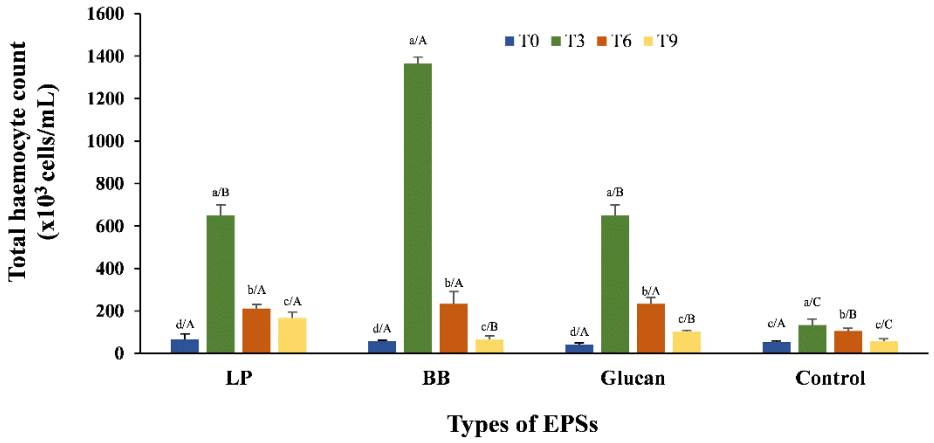

Figure 3 Total haemocyte count of *L. vannamei* fed EPS diets (T0: before the challenge; T3, T6, and T9 were 3, 6 and 9 days after the challenge, respectively). Different superscript uppercase letters indicate statistically significant differences between treatments at the same sample-collection time and different superscript lowercase letters indicate significant differences throughout time for each separate treatment by Duncan' test ($p < 0.05$)

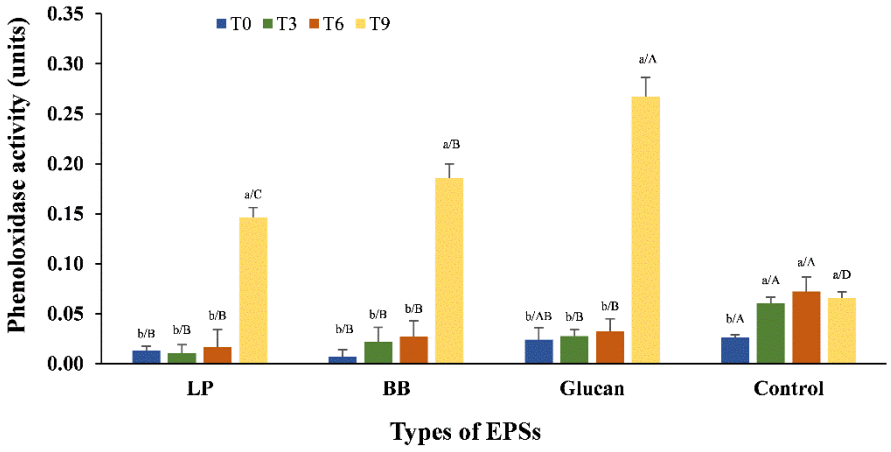

Figure 4 The phenoloxidase activity of *L. vannamei* fed EPS diets (T0: before the challenge; T3, T6, and T9 were 3, 6 and 9 days after the challenge, respectively). Different superscript uppercase letters indicate statistically significant differences between treatments at the same sample-collection time and different superscript lowercase letters indicate significant differences throughout time for each separate treatment by Duncan' test ($p < 0.05$)

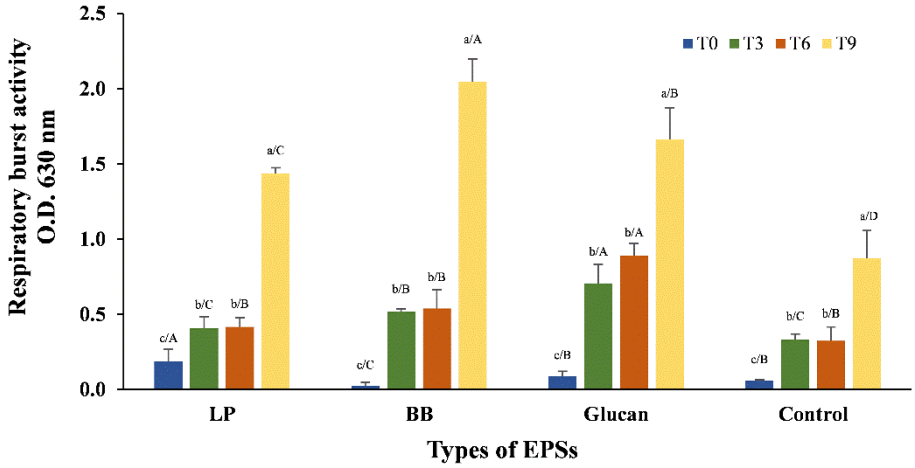

Figure 5 The respiratory burst activity of *L. vannamei* fed EPS diets (T0: before the challenge; T3, T6, and T9 were 3, 6 and 9 days after the challenge, respectively). Different superscript uppercase letters indicate statistically significant differences between treatments at the same sample-collection time and different superscript lowercase letters indicate significant differences throughout time for each separate treatment by Duncan' test ($p < 0.05$)

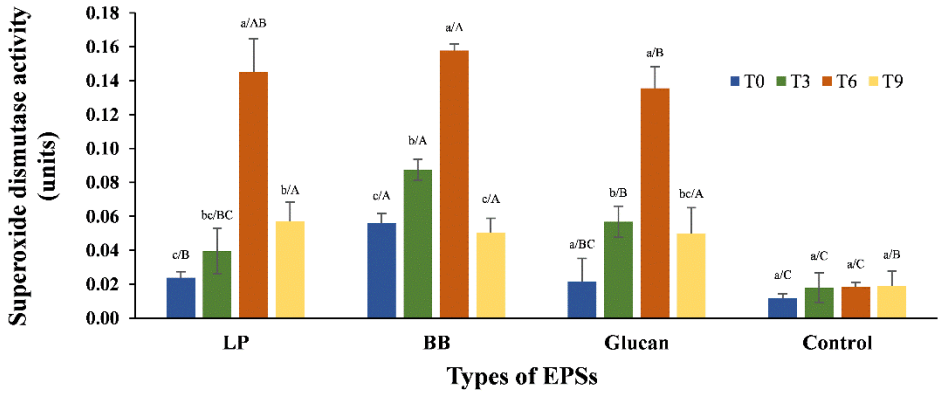

Figure 6 The superoxide dismutase activity of *L. vannamei* fed EPS diets (T0: before the challenge; T3, T6, and T9 were 3, 6 and 9 days after the challenge, respectively). Different superscript uppercase letters indicate statistically significant differences between treatments at the same sample-collection time and different superscript lowercase letters indicate significant differences throughout time for each separate treatment by Duncan' test ($p < 0.05$)

Cumulative mortality

The monitoring of shrimp mortality after the pathogen challenge revealed that the dietary application of LP, BB, and Glucan EPSs significantly decreased ($p<0.05$) the cumulative mortality of shrimp infected with *V. parahaemolyticus*, compared to the control. After 9 days of the challenge, the cumulative mortalities of the shrimp fed the diets containing LP, BB, and Glucan EPSs were 27%, 30%, and 39%, respectively. In contrast, the cumulative mortality of the control group was 91% (Figure 7). After 9 days of the challenge, the cumulative mortalities of the shrimp fed the diets containing LP, BB, and Glucan were 27, 30, and 39%, respectively. Meanwhile, the cumulative mortality of the control was 91% (Figure 7). These results indicate that the EPSs produced by LAB can enhance the ability of *L. vannamei* to resist and survive the infection by *V. parahaemolyticus*.

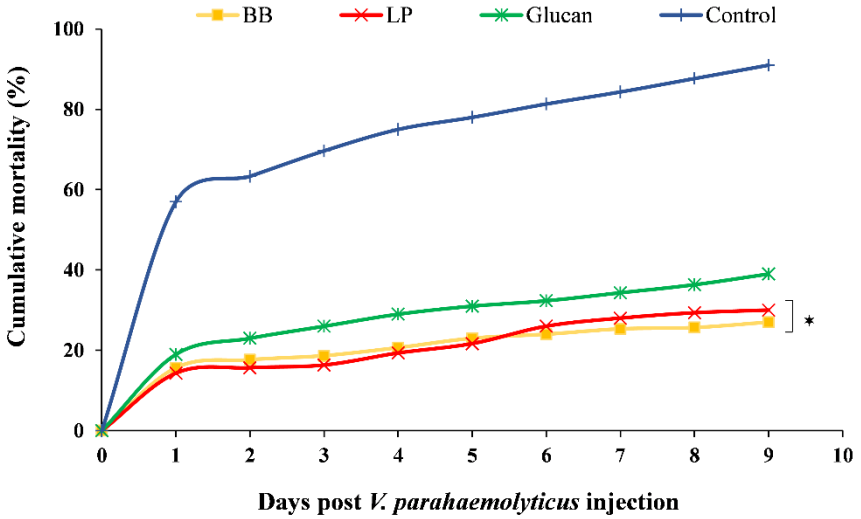

Figure 7 The cumulative mortality curve of *L. vannamei* fed EPS diets in the pathogen challenge. The asterisk represent significant differences between the LAB's EPS groups and the others by analyzing the Kaplan-Meier plot log-rank χ^2 test

DISCUSSION

Although many studies have indicated that EPSs from LAB have the prebiotic potential to stimulate growth performance in poultry (Ashfaq et al., 2020), this is the first study, to the best of our knowledge, to demonstrate their growth-promoting effects on the Pacific white shrimp, *L. vannamei*. Our results revealed significant differences in the SGR and shrimp survival between the EPS-supplemented groups and the control group. This is the first report on the effect of diets containing bacterial EPSs on the growth performance of *L. vannamei*. While there are no previous studies on the impact of bacterial EPSs on shrimp growth, several studies have demonstrated that dietary polysaccharides extracted from algae and plants could increase the growth performance of *L. vannamei* (Akbary and Aminikhoei, 2018; Su et al., 2020). Therefore, the findings of the current study suggest that the EPSs produced by LAB may have the potential to serve as prebiotics in aquaculture.

The population of LAB in the shrimp gut increased considerably with the addition of EPSs to the diet. This finding is consistent with a previous study that reported dietary fructo-oligosaccharides stimulated the growth of probiotics such as *Lactobacillus* sp. in the intestinal tract of *L. vannamei* (Zhou et al., 2007). In contrast to the increased LAB density, we observed a rapid decrease in the population of *V. parahaemolyticus* in the shrimp receiving the EPS-supplemented diets after the challenge. The inhibition of *V. parahaemolyticus* in shrimp by dietary polysaccharides has also been studied previously (Thanardkit et al., 2002; Elshopakey et al., 2018). EPSs can prevent pathogen adherence by operating as competing receptors in intestinal epithelial cells (Luna-González et al., 2012). In addition, EPSs also function as prebiotics by accelerating the growth of intestinal LAB (Grosu-Tudor et al., 2013), resulting in a reduction in the population of pathogenic bacteria. The immunomodulatory functions of prebiotics include stimulating the growth of anaerobic bacteria, specifically *Bifidobacterium*, preventing opportunistic/pathogenic adhesion, lowering the pH concentration of the colon by increasing the production of short-chain fatty acids (SCFAs), competing for nutrients, and up-regulating immune responses against infections (Mussatto and Mancilha, 2007; Luis, 2018).

Prebiotics have been reported to act as immunostimulants, which can directly impact the innate immune system of shellfish (Akhter et al., 2015). Therefore, the present study analyzed the immune parameters of shrimp to demonstrate the immune-enhancing effect of EPSs. It is well-established that increasing the total hemocyte count (THC) provides enhanced immune capability in crustaceans. Hemocytes are the key inducers of the cellular immune responses, including recognition, phagocytosis, and melanization, in shrimp (Bachère et al., 2004). Meanwhile, PO is the terminal enzyme of the prophenoloxidase (proPO) system, which is an important part of the shrimp immune system. PO activity is positively correlated with the shrimp's disease resistance capability (Wongprasert et al., 2014). Previous studies have indicated that dietary polysaccharides extracted from *Agave tequilana* (Luna-González et al., 2012), *S. cerevisiae* (Bai et al., 2010), and *S. commune* (Wang et al., 2008) substantially improve the innate immunity parameters of *L. vannamei*. Consistent with these earlier findings, our results showed that dietary supplementation of EPSs significantly increased the THC, PO activity, SOD activity, and respiratory burst of *L. vannamei* after the pathogen challenge. The increase in phagocytic activity, PO activity, and antibacterial ability in the EPS-supplemented groups further confirmed the beneficial effects of LAB-derived EPSs on the shrimp immune system.

The observed changes in THC, SOD activity, PO activity, and respiratory burst activity in shrimp challenged with *V. parahaemolyticus* could be explained by the immune response mechanisms triggered by the pathogen (Rajendran et al., 2022). The increase in THC at day 3 suggests an active recruitment and proliferation of hemocytes in response to the pathogen. The rise in SOD activity on day 6 reflects the shrimp's effort to combat oxidative stress generated by the infection (Harlina et al., 2024). Additionally, the increase in PO and respiratory burst activities on day 9 indicates a heightened immune activity, likely as a result of sustained infection and the need for enhanced defense mechanisms (Harlina et al., 2024). These immune markers are interrelated in the context of the shrimp's defense against *V. parahaemolyticus,* potentially achieved through sequential activation mechanisms. The increase in hemocytes initiates the immune response, leading to heightened SOD activity to manage oxidative stress. This is followed by an increase in respiratory burst activity as the shrimp attempt to eliminate the pathogen.

The immunostimulatory function of prebiotics is believed to involve their interaction with pattern recognition molecules, such as β-glucan and dectin-1 receptors, present on macrophages, which can activate signaling molecules like NF-kB and improve the capacities of immune cells (Brown et al., 2002; Yadav and Schorey, 2006). Prebiotics may also interact with Microbe-Associated Molecular Patterns (MAMPs), such as teichoic acids, peptidoglycans, glycosylated proteins, and bacterial capsule polysaccharides, to stimulate the immune response (Bron et al., 2011). An increase in the activities of SOD, PO, and respiratory burst has also been observed in *L. vannamei* fed a diet containing prebiotics such as mannan-oligosaccharides (MOS) (Hamsah et al., 2019), which is consistent with our findings. These results suggest that EPSs produced by LAB can be employed as an effective immunostimulant in shrimp aquaculture.

After the pathogen challenge, the health status and immune response performance of the shrimp were assessed by evaluating the cumulative mortality. *V. parahaemolyticus*, a hazardous pathogen, is known to cause Acute Hepatopancreatic Necrosis Disease (AHPND), or Early Mortality Syndrome (EMS), in *L. vannamei* (Khimmakthong and Sukkarun, 2017). Previous studies have demonstrated that the dietary intake of prebiotics could increase the resistance of Kuruma Shrimp (*Marsupenaeus japonicus*) against *V. parahaemolyticus* (Elshopakey et al., 2018). In the present study, shrimp fed with EPSs and subsequently inoculated with *V. parahaemolyticus* exhibited a 27-30% mortality rate, while the control group had a mortality rate of 91%. This reduced mortality is likely attributed to the enhanced immune response induced by the dietary

supplementation of EPSs. Furthermore, the EPSs increased the number of LAB in the shrimp gut, which may have enhanced the capacity of the beneficial bacteria to compete for adhesion sites with *V. parahaemolyticus* and other pathogens (Gomez-Gil et al., 2000). However, to fully elucidate the shrimp immune response after the *V. parahaemolyticus* challenge, further analysis of the expression of genes related to the immune response is required to explore the positive effect of EPSs on infected shrimp. Nevertheless, the current study offers valuable information on the immune-promoting mechanism of LAB-derived EPSs in shrimp.

CONCLUSIONS

The present study demonstrated the immunostimulatory effects of EPSs derived from LAB in the Pacific white shrimp, *L. vannamei*. The findings suggest that dietary supplementation of EPSs can significantly enhance the innate immune parameters of shrimp, including total THC, PO activity, SOD activity, and respiratory burst. The improved immune responses observed in the EPS-fed shrimp were further confirmed by the increased phagocytic activity, PO activity, and antibacterial ability, indicating the beneficial effects of LAB-derived EPSs on the shrimp immune system. The reduced cumulative mortality rate (27-30%) in shrimp challenged with the pathogen Vibrio parahaemolyticus, compared to the control group (91%), highlights the ability of EPSs to enhance the disease resistance of *L. vannamei*. The increase in the population of beneficial LAB in the shrimp gut may have contributed to the enhanced disease resistance by competing with *V. parahaemolyticus* for adhesion sites, thereby reducing the pathogen's colonization and proliferation. Overall, the findings of this study suggest that LAB-derived EPSs can be effectively utilized as a natural and eco-friendly immunostimulant in shrimp aquaculture to improve the health and disease resistance of *L. vannamei*.

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AUTHOR CONTRIBUTIONS

Nguyen Huu Thanh: Conceptualization, Methodology, Software. **Trinh Thi Lan, Nguyen Thi Thuy Hang, Nguyen Huu Yen Nhi**: Data curation, Writing-Original draft preparation. **Nguyen Phu Tho**: Visualization, Investigation, Writing-Reviewing, and Editing.

CONFLICT OF INTEREST

The authors report there are no competing interests to declare.

REFERENCES

Akbary, P., Aminikhoei, Z., 2018. Effect of polysaccharides extracts of algae Ulva rigida on growth, antioxidant, immune response and resistance of shrimp,

Litopenaeus vannamei against Photobacterium damselae. Aquac. Res. 49(7), 2503-2510.
Akhter, N., Wu, B., Memon, A., Mohsin, M., 2015. Probiotics and prebiotics associated with aquaculture: a review. Fish. Shellfish. Immunol. 45, 733-741.
Ashfaq, I., Amjad, H., Ahmad, W., Nasir, A., Ali, A., Ali, W.R., Khaliq, S., Hayat, A., Ali, H., Sattar, F., Ghauri, M.A., Anwar, M.A., 2020. Growth inhibition of common enteric pathogens in the intestine of broilers by microbially produced dextran and levan exopolysaccharides. Curr. Microbiol. 77(9), 2128-2136.
Bachère, E., Gueguen, Y., Gonzalez, M., De Lorgeril, J., Garnier, J., Romestand, B., 2004. Insights into the anti-microbial defense of marine invertebrates: the penaeid shrimps and the oyster Crassostrea gigas. Immunol. Rev. 198(1), 149-168.
Badel, S., Bernardi, T., Michaud, P.J.B.A., 2011. New perspectives for Lactobacilli exopolysaccharides. Biotechnol. Adv. 29(1), 54-66.
Bai, N., Zhang, W., Mai, K., Wang, X., Xu, W., Ma, H., 2010. Effects of discontinuous administration of β-glucan and glycyrrhizin on the growth and immunity of white shrimp Litopenaeus vannamei. Aquaculture. 306(1), 218-224.
Bardera, G., Usman, N., Owen, M., Pountney, D., Sloman, K.A., Alexander, M.E., 2018. The importance of behaviour in improving the production of shrimp in aquaculture. Rev. Aquac. 11(4), 1104-1132.
Beauchamp, C., Fridovich, I., 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44(1), 276-287.
Bell, K.L., Smith, V.J., 1993. In vitro superoxide production by hyaline cells of the shore crab Carcinus maenas (L.). Dev. Comp. Immunol. 17(3), 211-219.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72(1), 248-254.
Bron, P., Baarlen, P., Kleerebezem, M., 2011. Emerging molecular insights into the interaction between probiotics and the host intestinal mucosa. Nat. Rev. Microbiol. 10, 66-78.
Brown, G.D., Taylor, P.R., Reid, D.M., Willment, J.A., Williams, D.L., Martinez-Pomares, L., Wong, S.Y., Gordon, S., 2002. Dectin-1 Is A Major β-Glucan receptor on macrophages. J. Exp. Med. 196(3), 407-412.
Butt, U.D., Lin, N., Akhter, N., Siddiqui, T., Li, S., Wu, B., 2021. Overview of the latest developments in the role of probiotics, prebiotics and synbiotics in shrimp aquaculture. Fish. Shellfish. Immunol. 114, 263-281.
Chiu, C.H., Guu, Y.K., Liu, C.H., Pan, T.M., Cheng, W., 2007. Immune responses and gene expression in white shrimp, Litopenaeus vannamei, induced by Lactobacillus plantarum. Fish. Shellfish. Immunol. 23, 364-377.
Elshopakey, G.E., Risha, E.F., Abdalla, O.A., Okamura, Y., Hanh, V.D., Ibuki, M., Sudhakaran, R., Itami, T., 2018. Enhancement of immune response and resistance against Vibrio parahaemolyticus in kuruma shrimp (Marsupenaeus japonicus) by dietary supplementation of β-1,4-mannobiose. Fish. Shellfish. Immunol. 74, 26-34.
Garibay-Valdez, E., Martinez-Cordova, L.R., Lopez-Torres, M.A., Almendariz-Tapia, F.J., Martinez-Porchas, M., Calderon, K., 2020. The implication of metabolically active Vibrio spp. in the digestive tract of Litopenaeus vannamei for its post-larval development. Sci. Rep. 10(1), 11428.
Gomez-Gil, B., Roque, A., Turnbull, J.F., 2000. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. Aquaculture. 191(1), 259-270.
Grosu-Tudor, S., Zamfir, M., Meulen, R., Falony, G., Vuyst, L.C., 2013. Prebiotic potential of some exopolysaccharides produced by lactic acid bacteria. Rom. Biotechnol. Lett. 18, 8666-8676.

Hamprakorn, K., Maneewan, B., Jantasin, W., Panatuk, J., 2024. Effect of by-product from C-phycocyanin extraction as a prebiotic properties and probiotic microbial population. Vet. Integr. Sci. 23, 1-16.
Hamsah, H., Widanarni, W., Alimuddin, A., Yuhana, M., Junior, M.Z., Hidayatullah, D., 2019. Immune response and resistance of Pacific white shrimp larvae administered probiotic, prebiotic, and synbiotic through the bio-encapsulation of Artemia sp. Aquac. Int. 27(2), 567-580.
Harlina, H., Rosmiati, R., Hamdillah, A., Syahrul, S., Andriani, Y., 2024. Growth performance, non-specific immune activity, and resistance against Vibrio parahaemolyticus of whiteleg shrimp fed dietary Chromolaena odorata leaf flour components. Fish Aquatic Sci. 27, 434-446.
Hongpattarakere, T., Cherntong, N., Wichienchot, S., Kolida, S., Rastall, R.A., 2012. In vitro prebiotic evaluation of exopolysaccharides produced by marine isolated lactic acid bacteria. Carbohydr. Polym. 87(1), 846-852.
Hoseinifar, S.H., Soleimani, N., Ringø, E., 2014. Effects of dietary fructo-oligosaccharide supplementation on the growth performance, haemato-immunological parameters, gut microbiota and stress resistance of common carp (Cyprinus carpio) fry. Br. J. Nutr. 112(8), 1296-1302.
Hou, D., Huang, Z., Zeng, S., Liu, J., Wei, D., Deng, X., Weng, S., Yan, Q., He, J., 2018. Intestinal bacterial signatures of white feces syndrome in shrimp. Appl. Microbiol. Biotechnol. 102(8), 3701-3709.
Hu, X., Yang, H.-L., Yan, Y.-Y., Zhang, C.-X., Ye, J.-d., Lu, K.-L., Hu, L.-H., Zhang, J.-J., Ruan, L., Sun, Y.-Z., 2019. Effects of fructooligosaccharide on growth, immunity and intestinal microbiota of shrimp (Litopenaeus vannamei) fed diets with fish meal partially replaced by soybean meal. Aquac. Nutr. 25(1), 194-204.
Immanuel, G., Vincybai, V.C., Sivaram, V., Palavesam, A., Marian, M.P., 2004. Effect of butanolic extracts from terrestrial herbs and seaweeds on the survival, growth and pathogen (Vibrio parahaemolyticus) load on shrimp Penaeus indicus juveniles. Aquaculture. 236(1), 53-65.
Khimmakthong, U., Sukkarun, P., 2017. The spread of Vibrio parahaemolyticus in tissues of the Pacific white shrimp Litopenaeus vannamei analyzed by PCR and histopathology. Microb. Pathog. 113, 107-112.
Li, Y., Liu, H., Dai, X., Li, J., Ding, F., 2018. Effects of dietary inulin and mannan oligosaccharide on immune related genes expression and disease resistance of Pacific white shrimp, Litopenaeus vannamei. Fish. Shellfish. Immunol. 76, 78-92.
Luna-González, A., Almaraz-Salas, J.C., Fierro-Coronado, J.A., Flores-Miranda, M.D.C., González-Ocampo, H.A., Peraza-Gómez, V., 2012. The prebiotic inulin increases the phenoloxidase activity and reduces the prevalence of WSSV in whiteleg shrimp (Litopenaeus vannamei) cultured under laboratory conditions. Aquaculture. 362-363, 28-32.
Madhuri, K., Vidya Prabhakar, K., 2014. Microbial exopolysaccharides: biosynthesis and potential applications. Orient. J. Chem. 30, 1401-1410.
Maldonado Galdeano, C., Cazorla, S.I., Lemme Dumit, J.M., Vélez, E., Perdigón, G., 2019. Beneficial effects of probiotic consumption on the immune system. Ann. Nutr. Metabol. 74(2), 115-124.
Miller, R.A., Harbottle, H., 2018. Antimicrobial drug resistance in fish pathogens. Microbiol. Spectr. 6(1), 1-20.
Mussatto, S.I., Mancilha, I.M., 2007. Non-digestible oligosaccharides: a review. Carbohydr. Polym. 68(3), 587-597.
Nawaz, A., Javaid, A., Irshad, S., Hoseinifar, S.H., Xiong, H., 2018. The functionality of prebiotics as immunostimulant: Evidences from trials on terrestrial and aquatic animals. Fish. Shellfish. Immunol. 76, 272-278.
Neto, H., Nunes, A., 2015. Performance and immunological resistance of Litopenaeus vannamei fed a β-1,3/1,6-glucan-supplemented diet after per os

challenge with the Infectious myonecrosis virus (IMNV). Rev. Bras. de Zootec. 44, 165-173.

Nguyen, P.T., Nguyen, T.T., Nguyen, T.T.U., Hoang, Q.K., Nguyen, H.T., 2022. Improve the viability and extracellular polymeric substances bioactivity of Lactiplantibacillus plantarum VAL6 using the environmental adaptation. Food. Bioprod. Process. 131, 149-155.

Paul, P., Ramraj, S.K., Neelakandan, Y., Paari, K.A., Pattukumar, V., Arul, V., 2011. Production and purification of a novel exopolysaccharide from lactic acid bacterium Streptococcus phocae PI80 and its functional characteristics activity in vitro. Bioresour. Technol. 102, 4827-4833.

Poulose, G., 2011. Immune response of Penaeus monodon to the inactivated white spot syndrome virus preparation (Doctor of Philosophy). Cochin University of Science and Technology. Available online: http://hdl.handle.net/10603/3719.

Rajendran, K.V., Sreedharan, K., Deepika, A., Kulkarni, A., 2022. Shrimp immune system and immune responses. In: Makesh, M., Rajendran, K.V. (Eds.), Fish immune system and vaccines. Springer Nature Singapore, Singapore, pp. 17-43.

Sapkota, A., Sapkota, A.R., Kucharski, M., Burke, J., McKenzie, S., Walker, P., Lawrence, R., 2008. Aquaculture practices and potential human health risks: current knowledge and future priorities. Environ. Int. 34(8), 1215-1226.

Sestito, S., D'Auria, E., Baldassarre, M.E., Salvatore, S., Tallarico, V., Stefanelli, E., Tarsitano, F., Concolino, D., Pensabene, L., 2020. The role of prebiotics and probiotics in prevention of allergic diseases in infants. Front. Pediatr. 8, 583946.

Smith, V., Brown, J., Hauton, C., 2003. Immunostimulation in crustaceans: does it really protect against infection?. Fish. Shellfish. Immunol. 15, 71-90.

Su, C., Fan, D., Pan, L., Lu, Y., Wang, Y., Zhang, M., 2020. Effects of Yu-Ping-Feng polysaccharides (YPS) on the immune response, intestinal microbiota, disease resistance and growth performance of Litopenaeus vannamei. Fish. Shellfish. Immunol. 105, 104-116.

Thanardkit, P., Khunrae, P., Suphantharika, M., Verduyn, C., 2002. Glucan from spent brewer's yeast: Preparation, analysis and use as a potential immunostimulant in shrimp feed. World. J. Microbiol. Biotechnol. 18(6), 527-539.

Trujillo, L.E., Rivera, L., Hardy, E., Llumiquinga, E.M., Garrido, F., Chávez, J.A., Abril, V.H, País-Chanfrau, J.M., 2018. Natural strategies to improve growth and health of farmed shrimp. Available online: https://www.aquaculturealliance.org/advocate/ natural-strategies-growth-health-farmed-shrimp/

Van Doan, H., Hoseinifar, S.H., Faggio, C., Chitmanat, C., Mai, N.T., Jaturasitha, S., Ringø, E., 2018. Effects of corncob derived xylooligosaccharide on innate immune response, disease resistance, and growth performance in nile tilapia (oreochromis niloticus) fingerlings. Aquaculture. 495, 786-793.

Wang, Y.C., Chang, P.S., Chen, H.Y., 2008. Differential time-series expression of immune-related genes of Pacific white shrimp Litopenaeus vannamei in response to dietary inclusion of β-1,3-glucan. Fish. Shellfish. Immunol. 24(1), 113-121

Wongprasert, K., Rudtanatip, T., Praiboon, J., 2014. Immunostimulatory activity of sulfated galactans isolated from the red seaweed Gracilaria fisheri and development of resistance against white spot syndrome virus (WSSV) in shrimp. Fish. Shellfish. Immunol. 36(1), 52-60.

Yadav, M., Schorey, J., 2006. The β-glucan receptor Dectin-1 functions together with TLR2 to mediate macrophage activation by mycobacteria. Blood. 108, 3168-3175.

Yahfoufi, N., Mallet, J.F., Graham, E., Matar, C., 2018. Role of probiotics and prebiotics in immunomodulation. Curr. Opin. Food Sci. 20, 82-91.

Zhou, Q.C., Buentello, J.A., Gatlin, D.M., 2010. Effects of dietary prebiotics on growth performance, immune response and intestinal morphology of red drum (Sciaenops ocellatus). Aquaculture. 309(1), 253-257.

Zhou, Z., Ding, Z., Huiyuan, L.V., 2007. Effects of dietary short-chain fructooligosaccharides on intestinal microflora, survival, and growth performance of juvenile white shrimp, Litopenaeus vannamei. J. World Aquac. Soc. 38(2), 296-301.

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