EFFECTS OF SYNBIOTIC FROM NONI LEAF EXTRACT SUPPLEMENTATION ON SURVIVAL, GROWTH, AND RESISTANCE TO *Vibrio harveyi* IN THE WHITELEG SHRIMP, *Litopenaeus vannamei*

Thi Cam Tu Phan[1*], Truong Giang Huynh[1], Thi Kim Lien Nguyen[1] and Thi Thanh Hien Tran[1]

[1]College of Aquaculture and Fisheries, Can Tho University, Vietnam
[*]Corresponding author's Email: ptctu@ctu.edu.vn

ABSTRACT

This study assessed the effects of noni leaf and *Lactobacillus plantarum* CMT1 on survival, growth, and resistance to *Vibrio harveyi* of whiteleg shrimp, *Litopenaeus vannamei,* by improving digestive function and immunity. The experiment was conducted in a recirculating aquaculture system using a completely randomized design with five treatments and three replicates per treatment. One thousand five hundred healthy shrimp were assigned to 5 diet groups for a 60-day feeding period, including a control (commercial feed), diet 1 (commercial feed + 0.5% noni leaf), diet 2 (commercial feed + 1% noni leaf), diet 3 (commercial feed + 0.5% noni leaf + 10^8 CFU kg^{-1} of *L. plantarum* CMT1), and diet 4 (commercial feed + 1% noni leaf + 10^8 CFU kg^{-1} of *L. plantarum* CMT1). One-way ANOVA analysis showed that, compared with the control diet, other diets greatly enhanced growth indices of shrimp, with the highest growth observed in diet 3 ($p \leq 0.05$). Supplementation with diets 1, 2, 3, and 4 improved the abundance of *Lactobacillus* spp. and decreased *Vibrio* spp. in the shrimp intestine. Shrimp fed diets 1 and 3 showed significantly higher amylase activity than those in the control and in diets 2 and 4. In addition, shrimp fed diet 3 group showed greatly enhanced survival, increasing from 78.7% to 88.3%, and improved immune parameters after *Vibrio harveyi* challenge compared to other experimental diets and the control. In summary, the administration of 0.5% noni leaf and 10^8 CFU kg^{-1} of *L. plantarum* CMT1 increased growth performance, improved digestive function, and immunity system as well as promoted the survival rate against *V. harveyi* in shrimp.

Keywords: *Morinda citrifolia*, leaf extract, *Litopenaeus vannamei*, synbiotic, digestive enzyme

https://doi.org/10.36899/JAPS.2026.5.0104 Published first online June, 04, 2026

INTRODUCTION

Alongside pangasius, shrimp is one of the top two aquaculture products in Vietnam, with the majority of production concentrated in the Mekong Delta region. According to the General Statistics Office of Vietnam (2025), shrimp production was 1,000,080 tons in 2023, accounting for nearly 85% of the country's total shrimp output. Super-intensive whiteleg shrimp farming has rapidly expanded in many provinces in the Mekong Delta region because of its high expected profits; however, frequent and intensive extreme weather events are a major factor negatively affecting shrimp growth and health. To minimize antibiotic usage in aquaculture farming, synbiotic products are increasingly being developed and used as growth boosters and health-promoting agents for aquatic animals (Khanjani *et al*., 2024). The synergistic supplementation of probiotics and prebiotics (indigestible carbohydrates) like fructo-oligosaccharides (FOS), galacto-oligosaccharides, and mannan oligosaccharides has demonstrated beneficial effects on the host (Knipe *et al*., 2021). Recently, several studies have shown the advantageous effects of synbiotics on aquatic animals, particularly shrimp species. For example, Nababan *et al*. (2022) demonstrated *Pseudoalteromonas piscicida* combined with FOS enhanced the resistance of the *P. vannamei* to co-infection with White Spot Syndrome Virus (WSSV) and *Vibrio harveyi*, thereby improving shrimp development. In addition, Hong *et al*. (2022) reported that the administration of *Pediococcus pentosaceus* combined with FOS has demonstrated synergistic effects in enhancing growth, immunity, and resistance to *Vibrio parahaemolyticus* infection in whiteleg shrimp. According to Chen *et al*. (2020), the combination of mannan oligosaccharides and *Bacillus licheniformis* positively influences the overall growth, nutrient utilization, and immunological health of *P. vannamei*. Similar positive effects have been reported in other crustacean species supplemented with synbiotics in their diets, including prawn (*Macrobrachium rosenbergii*) (Li *et al*., 2024; Halim *et al*., 2018), brown shrimp (*P. aztecus*) (Kaya, 2025), and tiger shrimp (*P. monodon*) (Chin *et al*., 2025). Moreover, Ismail *et*

al. (2019) reported that synbiotics can be used as replacement for antibiotics for disease control in aquatic organisms, including Nile tilapia (*Oreochromis niloticus*). Recently, the utilization of natural plant extracts as prebiotics in combination with probiotics has emerged as a sustainable strategy for eco-friendly aquaculture farm management.

Plants, and their by-products, which are abundant in many biologically active phytochemicals, vitamins, minerals, and fibers, are used as a prebiotic that is extracted with different solvents and combined with animal feed, promoting health or stimulating growth (Mabrouki *et al*., 2017; Citarasu, 2010). Moreover, plant extracts have antimicrobial activity against various bacteria that cause diseases in aquatic animals (Citarasu, 2010; Bondad-Reantaso *et al.,* 2023). The noni tree (*Morinda citrifolia*) is used in traditional medicine and is widely cultivated in tropical regions, especially in Southeast Asia (Chan-Blanco *et al*., 2006). In Vietnam, this tree naturally grows in humid areas, particularly alongside canals and rivers. Mubarokah *et al.* (2023) reported that noni leaf extracts exhibit a diverse range of phytochemical constituents such as flavonoids, coumarins, terpenoids, and carotenoids. Moreover, noni leaf extract is rich in dietary fiber (24.53%) and polysaccharides (37.82%) (Nwakanma *et al*., 2022) that serve as a prebiotic, potentially enhancing the benefits of probiotics through synergistic effects, thereby improving nutrient digestibility and promoting growth in animals. In previous studies of diets supplemented with noni, noni levels in shrimp diets differ markedly depending on the plant parts used, including fruit, seed, and leaf, ranging from 1.25 to 10 g kg^{-1} of diet (Abidin *et al*., 2022; Phan *et al*., 2023). The probiotic *Lactobacillus plantarum* CMT1 at 10^8 CFU kg^{-1} diet in shrimp feed is the optimal dose to promote weight gain and digestion and absorption, which can modulate intestinal microorganisms and could consequently enhance production efficiency of whiteleg shrimp (Phan *et al*., 2024). To develop synbiotics from natural plants or their by-products as prebiotic components in aquaculture species, optimization of prebiotic and probiotic concentration is a key prerequisite for their practical and effective application. Limited research has addressed the potential of probiotics and noni leaf extracts to promote aquatic animal growth and health, especially in strengthening resistance to pathogenic bacteria. Previous studies have not consistently demonstrated the synergistic effects of noni leaf extract and *L. plantarum* in the whiteleg shrimp. Therefore, this study evaluated the effect of prebiotics, noni leaf extract plus *L. plantarum* CMT1 probiotics, on weight gain, digestive enzymes, intestinal bacteria population, and resistance against pathogenic bacteria in whiteleg shrimp. The present study also underscores the prospective effects of synbiotics and determines the optimal dosage for their applications in shrimp farming.

MATERIALS AND METHODS

Experimental diets: Noni leaf extract was prepared using the procedure described by Phan *et al.* (2023). Briefly, dried noni leaf powder was extracted with 70% methanol for 48 h. The filtered liquid was then concentrated and stored at 4°C until further use in experiments. *Lactobacillus plantarum* CMT1 was provided by the Laboratory of Probiotics, Can Tho University, Can Tho city, Vietnam. Bacteria were cultured in glass tubes without shaking in MRS (De Man, Rogosa and Sharpe) medium (Himedia, India) at 37°C. The bacteria were adjusted to a concentration of 10^9 CFU mL^{-1} using sterile sodium chloride (0.9%). The noni leaf extract and diluted bacterial suspension were thoroughly incorporated into commercial feed to prepare the shrimp diets for different experimental groups. After preparation, all diets, including the control diet, were uniformly coated with 0.5% binder (Aqua Vina, Vietnam) to ensure consistent pellet stability, then dried and stored at 4°C until use.

Experiment design: The experiment was conducted from February to April, 2025 at the wet laboratory, College of Aquaculture and Fisheries, Can Tho University, Vietnam. Post-larval shrimp were sourced from a commercial hatchery and acclimatized for two weeks. The experiment was designed as a completely randomized design (CRD) with three replicates per treatment. A total of 1500 shrimp juveniles (average weight: 0.6 ± 0.18 g) were divided into 5 experimental groups, with three replicates of 100 shrimp each (300 shrimp per group). The different diet groups included (1) control; (2) diet 1 (0.5% noni); (3) diet 2 (1% noni); (4) diet 3 (0.5% noni + 10^8 CFU kg^{-1} *L. plantarum* CMT1); (5) diet 4 (1% noni + 10^8 CFU kg^{-1} *L. plantarum* CMT1). The commercial feed used in this study was sourced from GrowMax group (Vietnam) and contained 40 - 43% protein and 6% lipid. Shrimp were stocked into 500-L composite tanks equipped with a recirculation system and continuous aeration. Different diets were provided four times per day during the 60-day experimental period. Feed intake was recorded for each replicate by collecting uneaten feed, drying it to constant weight, and subtracting it form the total feed provided. . Throughout the culture period, minerals were added weekly to all treatments. Daily measurements and recordings were made using a Handheld Multiparameter (YIS model, USA), and the culture conditions were 26.8 - 27.6°C for temperature, 7.6 - 7.9 for pH, and DO 5.1 - 5.7 mg L^{-1}. Alkalinity, ammonium nitrogen, and nitrite were analyzed weekly (APHA, 2017), and the values were in the range of 102.7 - 150.9 mg$CaCO_3$ L^{-1}, 0.106 - 0.304 mg L^{-1}, and 0.101 - 0.337 mg L^{-1}, respectively.

Challenge analysis: After the 60-day feeding period, shrimp of all groups were injected with pathogenic *V. harveyi*. Bacteria were adjusted to 10^8 CFU mL^{-1} and *V. harveyi* was cultured according to a standard procedure provided by Phan

et al. (2025). The challenge dose was based on the median lethal dose (LD_{50}), which was approximately 5.0×10^7 CFU mL^{-1} at 96 h post-challenge, as determined in a preliminary test using the Reed and Muench method, and was selected to induce approximately 50% total mortality (data not shown). Each shrimp was injected with 20 µL of 5.0×10^7 CFU mL^{-1} bacterial suspension into the second abdominal segment. A total of sixty shrimp were randomly selected from each diet group (20 shrimp per replicate tank). Shrimp in the control were also injected with an equivalent level of NaCl (0.85%) as the negative control. During 96 hrs, dead shrimp of each group were monitored and documented, and nine shrimp in the diet group (3 shrimp per replicate tank) were collected to measure total hemocyte count (THC), phenoloxidase (PO), and hemocyanin.

Growth performance: After a 60-day culture period, shrimp growth performance and feed conversion ratio (FCR) were evaluated. Individual body weight was measured using an electronic analytical balance (± 0.01 g accuracy, model: XY600-2C, XingYun, China). The individual survival in each replicate was counted manually, and FCR, defined as the total feed consumed divided by the weight gain, was calculated. Feed intake was determined by subtracting the uneaten feed from the amount of feed offered. Growth parameters were determined as follows:

Weight gain (WG, g) = final body weight – initial body weight

Specific growth rate (SGR) (% day^{-1}) = ([Ln final weight - Ln initial weight]/ days) × 100

Daily weight gain (DWG) = (Final weight – Initial weight)/ days

Survival rate (%) = (number at harvest/number at stocking) × 100

FCR = feed intake/WG

Biomass (kg m^{-3}) = (mean final weight × final number of shrimp)/ volume of water

Digestive enzyme and intestinal bacteria analysis: For digestive enzyme and intestinal bacterial analyses, five shrimp per replicate were randomly sampled. Following the previously reported method by Phan *et al.* (2024), protease and amylase were measured according to the methods of Lowry *et al.* (1951) and Bernfeld (1955), respectively. For leu-aminopeptidase determination, the enzyme parameters were analyzed following the procedure outlined in Ezquerra *et al.* (1999). Enzyme activity was expressed as U mg $protein^{-1}$, where one unit (U) is defined as the amount of enzyme required to catalyze the conversion of 1 µmol of substrate per minute under the assay conditions. Each measurement included three replicates.

To minimize the number of animals used, the same individual shrimp was used for total bacteria, *Vibrio* spp., and *Lactobacillus* spp. counts, and immune parameters. The intestines were collected, weighed, and homogenized in 0.85% NaCl. Then, 100 µL of the intestinal homogenate was plated on TSA, MRS, and TCBS agar petri dishes to measure the total bacteria, *Lactobacillus* spp., and *Vibrio* spp. counts, respectively. The plates were stored at 30◦C for TSA and TCBS, and at 37◦C for MRS for 24 - 48 h, and density was expressed as CFU g^{-1}.

Analysis of immune parameters: THC was assessed based on the procedure outlined in Le Moullac *et al.* (1997), with minor change. Shrimp hemolymph was drawn and immediately added to an anticoagulant solution (pH 7.55). Hemocytes were counted under a microscope at 40× magnification. PO was measured using 3 mg mL^{-1} L-dihydrophenylalanine (L-DOPA) as the substrate (Giang *et al.,* 2011). Hemocyanin in hemolymph was detected following the procedure reported by Pascual *et al*. (2003) and Hagerman (1983).

Data analysis: All results are reported as means ± standard errors (SE). The data met the assumptions of normality and homogeneity of variances, as confirmed by Shapiro-Wilk and Levene's tests, respectively. Data were analyzed using one-way ANOVA. When significant differences were detected, means were separated using Tukey's honestly significant difference (HSD) test as a post hoc analysis. All statistical analyses were performed using SPSS (version 22.0). Differences were considered significant at $p \leq 0.05$.

RESULTS

Growth performance and survival: At the end of the 60-day feeding trail, growth parameters were significantly higher in shrimp fed diets 1, 2, 3, and 4 (four diets) compared to the control group (Table 1). Specifically, the final weight, weight gain, and DWG values were greatest for diet 3 group and reported significant differences compared with other diets ($p \leq 0.05$). Shrimp fed diet 3 exhibited the highest SGR, which was significantly higher than that of shrimp fed diet 1, diet 2, and the control ($p \leq 0.05$). Shrimp survival rate increased, and FCR values decreased in four diet groups compared with those in the control diet ($p \leq 0.05$). No statistical significances in survival rates were observed among diets 1-4.

Table 1. Growth performance of the whiteleg shrimp fed different diets for 60 days

Parameters	Treatments				
	Control	Diet 1	Diet 2	Diet 3	Diet 4
Initial weight (g)	0.62±0.005^{a}	0.62±0.009^{a}	0.62±0.009^{a}	0.62±0.009^{a}	0.62±0.014^{a}
Final weight (g)	16.5±0.12^{a}	17.69±0.12^{b}	17.34±0.03^{b}	18.31±0.14^{c}	17.72±0.1^{b}
Weight gain (WG, g)	15.88±0.12^{a}	17.06±0.12^{b}	16.72±0.03^{b}	17.69±0.14^{c}	17.1±0.09^{b}
Daily weight gain (DWG, g day^{-1})	0.245±0.002^{a}	0.261±0.002^{b}	0.256±0.004^{b}	0.269±0.001^{c}	0.261±0.005^{b}
Specific growth rate (SGR, % day^{-1})	5.011±0.007^{a}	5.056±0.017^{b}	5.039±0.017^{ab}	5.105±0.021^{c}	5.074±0.029^{bc}
Survival rate (%)	78.7±1.45^{a}	86.0±0.58^{b}	85.3±0.88^{b}	88.3±0.67^{b}	86.7±1.45^{b}
Biomass (kg m^{-3})	2.62±0.148^{a}	2.93±0.076^{ab}	3.14±0.03^{b}	3.25±0.04^{b}	3.25±0.04^{b}
Feed conversion ratio (FCR)	1.34±0.03^{a}	1.19±0.03^{b}	1.21±0.02^{b}	1.12±0.02^{b}	1.20±0.02^{b}

Data are expressed as mean ± SE.
Different letters (a, b, c) within the row indicate significant differences ($p \leq 0.05$).

Digestive enzyme activity and bacterial count: The levels of digestive enzyme activity in all diet groups are shown in Fig. 1. Amylase level was significantly greater in the diet 1 and 3 groups compared with the other groups ($p \leq 0.05$) (Fig. 1a). All the experimental diets, except diet 2, resulted in higher protease activity compared to the control diet ($p \leq 0.05$); however, no significant variation was detected among diets 1, 3, and 4 (Fig. 1b). The diet 3 group exhibited the highest leu-aminopeptidase activity, while no significant differences were observed among diets 1, 2, and 4 (Fig. 1c).

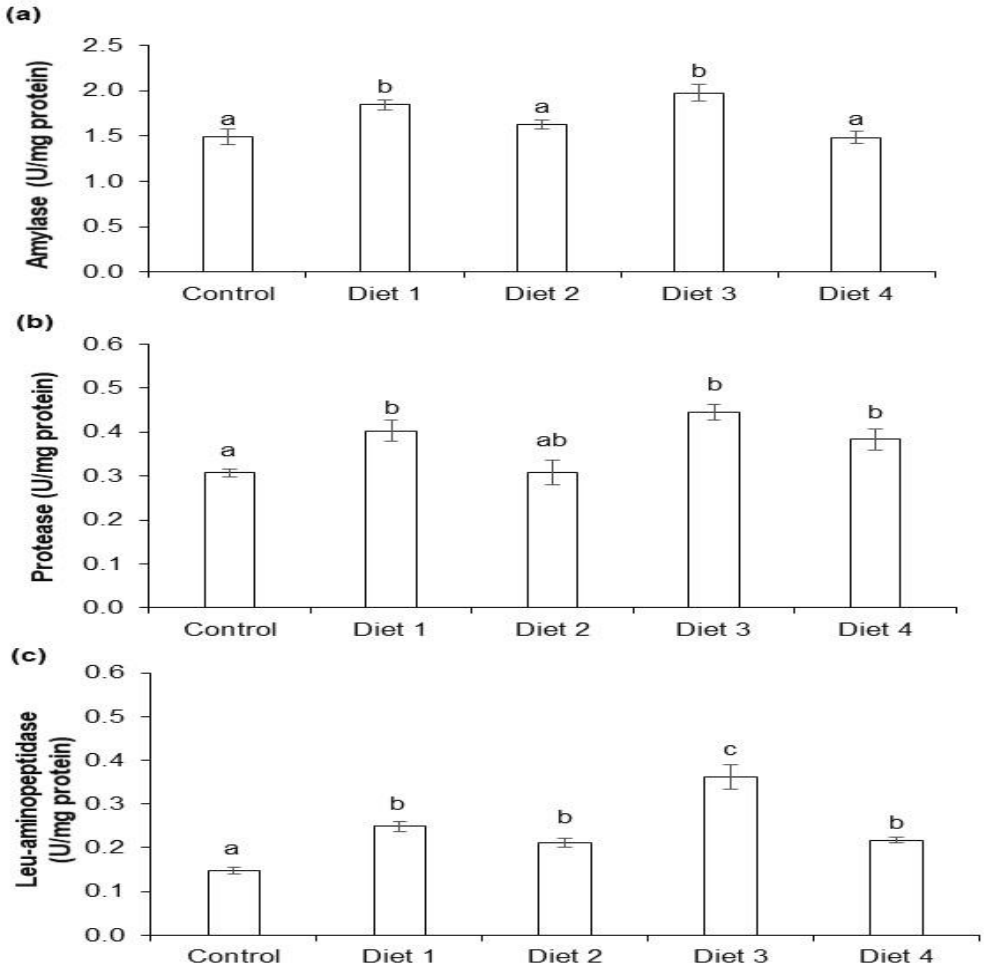

Fig. 1. Digestive enzyme activity of Amylase (a), Protease (b), and Leu-aminopeptidase (c) (U mg protein^{-1}) in the shrimp's intestine.

^{abcd}Different letters indicate significant differences among treatments ($p \leq 0.05$).

Intestinal bacteria population data are shown in Fig. 2. Supplementation with four different diets had no significant effect on total bacteria density ($p > 0.05$), except for the diet 3. Shrimp in the experimental groups exhibited significantly reduced *Vibrio* spp. densities in the intestine than the control diet ($p \leq 0.05$). The opposite result was found *Lactobacillus* spp. density, in which the densities in the diets 1, 2, 3, and 4 showed greatly higher population than the control group ($p \leq 0.05$).

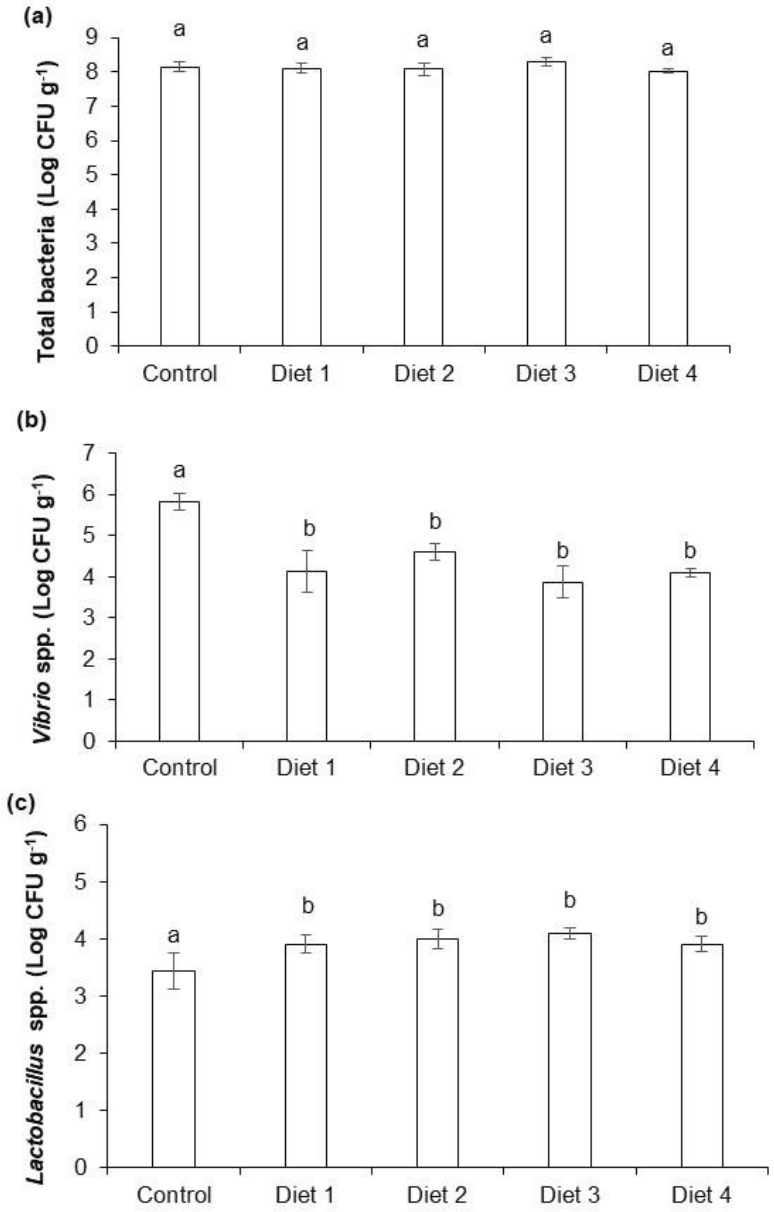

Fig. 2. Density of total bacteria (a), *Vibrio* spp. (b), and *Lactobacillus* spp. (c) counts in the intestine of whiteleg shrimp after 60 days of feeding experimental diets.

[abcd]Different letters above the bar indicate significant differences between treatments ($p \leq 0.05$).

Capacity for resistance to *Vibrio harveyi*: The result of the one-way ANOVA showed that the survival rates in the diet 1, 2, 3, and 4 groups were markedly greater compared with the positive control ($p \leq 0.05$) (Fig. 3). However, insignificant differences were found between diets 1, 2, 3, and 4 groups ($p > 0.05$). The negative control group exhibited no mortality, indicating the test conditions did not adversely affect shrimp survival.

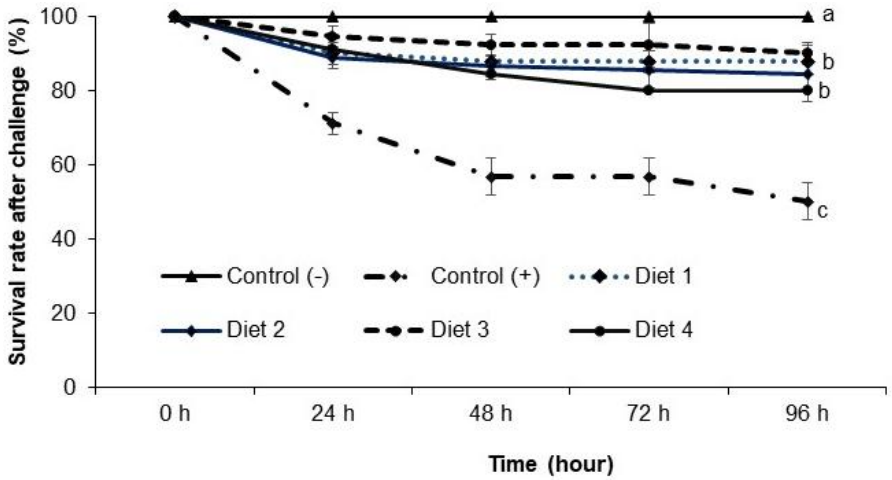

Fig. 3. Survival rate of the whiteleg shrimp at 96 h post-challenge with *Vibrio harveyi*.
Different letters (a, b, c) indicate significant differences between treatments ($p \leq 0.05$).

Immune indicators of whiteleg shrimp after challenge with *V. harveyi* are presented in Fig. 4. The diet 3 group exhibited the highest THC level, which was significantly higher than that of the control group and the other three diet groups ($p \leq 0.05$). Similarly, the diet 3 group also showed the greatest values for PO and haemocyanin (Fig. 4b, c), which were greater than in the remaining groups ($p \leq 0.05$). Overall, the immune response was markedly higher in the group fed diet 3, including 0.5% noni plus *L. plantarum* CMT1, compared with the groups fed diets supplemented with noni alone ($p \leq 0.05$).

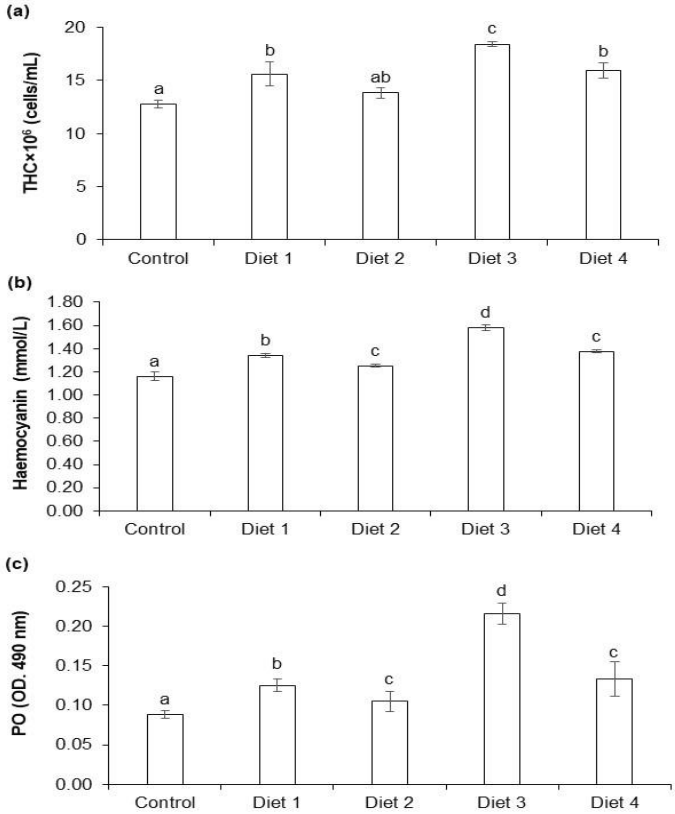

Fig. 4. Immune parameters including Total haemocyte count, THC (a), Hemocyanin (b), and Phenoloxidase, PO (c) in the whiteleg shrimp.
*abcd*Different letters indicate significant differences among treatments ($p \leq 0.05$).

DISCUSSION

Several studies have shown that synbiotics can serve as alternatives to antibiotics, resulting in higher yields and improved sustainability in shrimp aquaculture. In recent years, synbiotics developed from prebiotics - derived from plants and fibers - have been widely recognized in shrimp farming. Notably, plant extracts might serve as prebiotics, supplying substrates for probiotic growth (Kaur *et al*., 2021; Zhang *et al*., 2024), which consequently increases growth performance of aquatic animals. Moreover, these extracts metabolized by probiotics are typically absorbed and utilized more efficiently by the host. This study clearly showed that diet administration of either noni leaf alone or combined with probiotic *L. plantarum* led to improved growth performance in whiteleg shrimp. In addition, the synbiotic treatment containing 0.5% noni leaf extract and *L. plantarum* produced higher growth performance than diets supplemented with noni leaf extract alone, suggesting a synergistic interaction between the plant extract and the probiotic. This result agrees with the report of Abidin *et al*. (2022), who demonstrated that the combining *Moringa oleifera* leaf extract with *L. acidophilus* in the diet resulted in greater weight gain (595.2 ± 11.6%) in whiteleg shrimp than either supplementation alone (459.0 ± 23.4%). Previous studies have shown that increased digestive enzyme activity and higher total intestinal bacterial counts can enhance feed utilization efficiency, thereby supporting growth (Abdel-Ghany *et al*., 2020; Khanjani *et al*., 2024). A 60-day feeding trial with noni leaf extract either alone or in combination with *Lactobacillus plantarum* CMT1 increased the survivability in *L. vannamei*, likely due to the regulation of intestinal microbial population by the prebiotic and synbiotic treatments.

Shrimp intestinal bacteria are essential for maintaining shrimp health and overall well-being by improving digestion and nutrient absorption, protecting against pathogens, and regulating immunity (Holt *et al*., 2021). As indicated by the findings of this study, the addition of noni leaf extract or its combination with *L. plantarum* CMT1 significantly decreased *Vibrio* density and increased *Lactobacillus* spp. population in shrimp intestine. These findings suggest that synbiotic supplementation effectively modulates the microorganism flora in the shrimp intestines. Similarly, the report of Obeng-Boateng *et al.* (2024) indicated that bioactive compounds of noni leaf are natural antibiotics and can suppress pathogenic bacteria. In addition, noni leaves are rich in carbohydrates (Singh *et al*., 2008), which can serve as a substrate to promote the proliferation of probiotics. These probiotics produce organic acids during fermentation, thereby reducing the intestinal pH (Hassan *et al*., 2020) and creating unfavorable conditions for pathogenic organisms, such as *Vibrio* spp. Another explanation is that prebiotics could promote the proliferation of beneficial bacteria while suppressing harmful species by competing with glycoconjugates on the intestinal epithelial surface. This interaction enhances mucus secretion and increases the synthesis of short-chain fatty acids and cytokines, contributing to enhanced gut health and immune responses (Hoseinifar *et al*., 2017). The probiotic component, *L. plantarum* in synbiotics produces antimicrobial compounds, such as bacteriocins, which inhibit growth of pathogens on the intestinal wall and help reinforce the mucosal barrier (Echegaray *et al*., 2023). Prabawati *et al.* (2022) demonstrated that synbiotic-supplemented diets for whiteleg shrimp significantly increased *Lactobacillus* sp. with a concurrent decrease in *Vibrio* genus bacteria in the digestive tract. Moreover, Chin *et al*. (2025) revealed that administration of synbiotics derived from *L. plantarum* L20 and *Sargassum polycystum* significantly increased the abundance of Lactobacillaceae in the black tiger shrimp intestines. This finding indicated that supplementation of 0.5-1% noni leaf extract combined with *L. plantarum* CMT1 through the diet markedly increased the total *Lactobacillus* population in the gastrointestinal tract of *L. vannamei*.

The findings also showed that enzyme activity in shrimp fed with a synbiotic-supplemented diet plus 0.5% noni leaf extract was greater than that in shrimp fed the remaining diets. These findings suggest a synergistic interaction between noni leaf extract and *L. plantarum* CMT1 that enhances digestive function and supports nutrient absorption and growth performance. This may be explained by phytochemicals in noni leaves that enhance the probiotic activity of *L. plantarum* by acting as selective growth-promoting substrates and quorum-sensing modulators. They may also exert antimicrobial effects that suppress competing pathogens, thereby improving gut microbial balance. However, higher inclusion levels of plant extracts may introduce excessive bioactive compounds, such as phenolics, tannins, or other secondary metabolites, which could negatively affect feed palatability, digestive processes, or nutrient utilization. Therefore, a moderate level (0.5%) of noni leaf extract may provide an optimal balance of beneficial compounds, while higher levels (1%) may reduce its positive effects. Notably, dietary administration of synbiotic also showed the highest levels of proteases and leu-aminopeptidase, which are vital for protein digestion and assimilation. Similarly, Pardede *et al*. (2024) showed that synbiotic supplementation enhanced amylase and protease levels, resulting in increased digestive system and whiteleg shrimp growth. Abidin *et al.* (2022) showed significant enhancement in intestinal enzyme activities in *L. vannamei* fed a mixture of *L. acidophilus* and *Moringa oleifera* leaf. Similarly, Hasyimi *et al*. (2020) observed that a combination of 0.5% honey and *Bacillu*s sp. greatly influenced protease, amylase, and lipase activities in the gut of the whiteleg shrimp. These improvements in digestive enzyme production are likely associated with the increased abundance of beneficial bacteria in the intestine, which could produce exogenous enzymes and potentially stimulate endogenous enzyme activity (Maas *et al*., 2021; Rahayu *et al*., 2024).

Dietary supplementation of noni leaf extract individually or mixed with *L. plantarum* CMT1 improved the defensive responses against infection with *V. harveyi* (10^8 CFU mL^{-1}) in the whiteleg shrimp. Several studies have demonstrated reductions in THC and PO activity in whiteleg shrimp after pathogen injection (Ekasari *et al.,* 2014; Wang and Chen, 2005). In this study, although THC, PO, and hemocyanin decreased after the challenge test in all groups, shrimp fed noni leaf extract alone or in combination with *L. plantarum* CMT1 showed higher levels compared to the control group. This finding is partially consistent with the observation of Oktaviana *et al*. (2014), who demonstrated the administration of synbiotics in shrimp diet markedly increased THC and PO after co-injection of IMNV and *V. harveyi*. The enhancement in survival from the experimental groups was associated with an increase in PO and THC values compared with the control group. Previous studies have reported that increased immune responses to various infections are due to enhanced THC and PO level, which promote protection against pathogens, thereby reducing mortality (Oktaviana *et al*., 2014; Zubaidah *et al*., 2015). The highest levels of THC, hemocyanin, and PO were recorded in whiteleg shrimp fed 0.5% noni leaf extract combined with *L. plantarum* CMT1 at 10^8 CFU kg^{-1}, which indicates a dose-dependent effect of the diet on the host's immune response.

The current findings indicate that the supplementation of noni leaf extract, either alone or in combination with *L. plantarum* CMT1, enhances digestive enzyme activities in whiteleg shrimps, leading to better growth performance, survival, and feed conversion ratios. Supplementation with a synbiotic formulation containing 0.5% noni leaf extract and *L. plantarum* CMT1 at 10^8 CFU kg^{-1} is recommended to promote overall growth rate, feed utilization, and disease resistance in shrimp culture. As noni leaves are a readily available plant resource in Vietnam, their use as a low-cost, locally sourced prebiotic offers practical potential for aquaculture applications. Overall, these results provide a theoretical basis for utilizing plant products in the development of cost-effective prebiotics and synbiotics, thereby supporting sustainable aquaculture and advancing the circular economy. Future studies are needed to optimize dosage and evaluate efficacy under field conditions.

Acknowledgments: This work was supported by the Vingroup Innovation Foundation (VINIF) through the Postdoctoral Scholarship Programme, code VINIF.2024.STS.33, awarded to Phan Thi Cam Tu.

REFERENCES

Abidin, Z., H. T. Huang, Y. F. Hu, J. J. Chang, C.Y. Huang, Y. S. Wu and F. H. Nan (2022). Effect of dietary supplementation with *Moringa oleifera* leaf extract and *Lactobacillus acidophilus* on growth performance, intestinal microbiota, immune response, and disease resistance in whiteleg shrimp (*Penaeus vannamei*). Fish Shellfish Immunol. 127: 876-890. https://doi.org/10.1016/j.fsi.2022.07.007.

Abdel-Ghany, H. M., M. E. Salem, S. S. Abouelkhier and A. M. Helal (2020). Effect of a cocktail of enzymes and probiotics on the growth and the bacterial enumeration in gut and effluents of red tilapia (*Oreochromis niloticus* × *O. Mossambicus*). Egypt. J. Aquat. Res. 46: 289-294. https://doi.org/10.1016/j.ejar.2020.07.001.

APHA. (2017). Standard methods for the examination of water and wastewater, 23th edition, American Public Health Association 800 I Street, NW, Washington DC, 1504 p.

Bernfeld, P. (1955). Amylases, α and β. In: Abelson J, Simon M, Verdine G, Pyle S (Eds.), Methods in Enzymology, Academic Press, pp. 149-158.

Bondad-Reantaso, M. G., B. MacKinnon, I. Karunasagar, S. Fridman, V. Alday-Sanz, E. Brun, M. L. Groumellec, A. Li, W. Surachetpong, I. Karunsagar, B. Hao, A. Dall'Occo, R. Urbani and A. Caputo. (2023). Review of alternatives to antibiotic use in aquaculture. Rev. Aquacult. 15(4): 1421-1451. http://doi.org/10.1111/ raq.1278.

Chan-Blanco, Y., F. Vailant, A. M. Perez, M. Reynes, J. Brillouet and P. Brat (2006). The noni fruit (*Morinda citrifolia* L.): A review of agricultural research, nutritional and therapeutic properties. J. Food Compos. Anal. 19(6-7): 645-654. https://doi.org/10.1016/j.jfca.2005.10.001

Chen, M., X. Q. Chen, L. X. Tian, Y. J. Liu and J. Niu (2020). Beneficial impacts on growth, intestinal health, immune responses and ammonia resistance of pacific white shrimp (*Litopenaeus vannamei*) fed dietary synbiotic (mannan oligosaccharide and *Bacillus licheniformis*). Aquac. Rep. 17:100408. https://doi.org/10.1016/j.aqrep.2020.100408

Chin, Y. K., M. Azzam-Sayuti, A. Mohamad, A. O. Haifa-Haryani, M. I. Ahmad, M. F. Nazarudin, F. S. M. Ali, M. I. Ida-Muryany Karim and S. Anna (2025). Elucidation of synbiotic diet comprising of *Lactobacillus plantarum* L20 and *Sargassum polycystum* on gastrointestinal microbiota, tissue structures and AHPND associated dysbiosis susceptibility in black tiger shrimp (*Penaeus monodon*). Aquaculture 594:741339. https://doi.org/10.1016/j.aquaculture.2024.741339.

Citarasu, T. (2010). Herbal Biomedicines: a new opportunity for aquaculture industry. Aquac. Int. 18: 403-414. https://doi.org/10.1007/s10499-009-9253-7.

Echegaray, N., B. Yilmaz, H. Sharma, M. Kumar, M. Pateiro, F. Ozogul and J. M. Lorenzo (2023). A novel approach to *Lactiplantibacillus plantarum:* From probiotic properties to the omics insights. Microbiological Research 268:127289. https://doi.org/10.1016/j.micres.2022.127289.

Ekasari, J., M. H. Azhar, E. H. Surawidjaja, S. Nuryati, P. De Schryver and P. Bossier (2014). Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources. Fish Shellfish Immunol. 41(2): 332-339. https://doi.org/10.1016/j.fsi.2014.09.004.

Ezquerra, J. M., F. L. Garcia-Carreno, A. M. Guillermo and N. F. Haard (1999). Effect of feed diet on aminopeptidase activities from the hepatopancreas of white shrimp (*Penaeus vannamei*). Journal of Food Biochemistry 23: 59-74. https://doi.org/10.1111/j.1745-4514.1999.tb00005.x.

Giang, H. T., S. T. Yeh, Y. L. Lin, J. F. Shyu, L. L. Chen and J. C. Chen. (2011). White shrimp *Litopenaeus vannamei* immersed in seawater containing *Sargassum hemiphyllum* var. chinense powder and its extract showed increased immunity and resistance against *Vibrio alginolyticus* and white spot syndrome virus. Fish Shellfish Immunol. 79: 284-293. https://doi.org/10.1016/j.fsi.2011.05.014.

Hagerman, L. (1983). Haemocyanin concentration of juvenile lobsters (*Homarus gammarus*) in relation to moulting cycle and feeding conditions. Marine Biology 77: 11-17. https://doi.org/10.1007/BF00393205.

Halim, A. M., A. Prajitno and C. C. Chang (2018). Enhancement glutathione peroxidase activity and 2-macroglubulin gene expression of *Macrobrachium rosenbergii* fed with aqueous *Morinda citrifolia* leaves extract-supplemented diet. Indonesian Journal of Tropical Aquatic 1(1): 9-16. https://doi.org/10.22219/ijota.v1i1.4849

Hassan, M. U., H. Nayab, T. U. Rehman, M. P. Williamson, K. U. Haq, N. Shafi and F. Shafique (2020). Characterisation of bacteriocins produced by *Lactobacillus* spp. Isolated from the traditional Pakistani yoghurt and their antimicrobial activity against common foodborne pathogens. BioMed Research International 2020: 1-10. https://doi.org/10.1155/2020/8281623

Hasyimi, W., W. Widanarni and M. Yuhana (2020). Growth performance and intestinal microbiota diversity in pacific white shrimp *Litopenaeus vannamei* fed with a probiotic bacterium, honey prebiotic, and synbiotic. Curr. Microbiol. 77(10): 2982-2990. https://doi.org/10.1007/s00284-020-02117-w.

Holt, C. C., D. Bass, G. D. Stentiford and M. V. D Giezen (2021). Understanding the role of the shrimp gut microbiome in health and disease. Journal of Invertebrate Pathology 186: 107387. https://doi.org/10.1016/j.jip.2020.107387

Hong, N. T. X., N. T. H. Linh, K. Baruah, D. T. B. Thuy and N. N. Phuoc (2022). The combined use of *Pediococcus pentosaceus* and fructooligosaccharide improves growth performance, immune response, and resistance of Whiteleg shrimp *Litopenaeus vannamei* against *Vibrio parahaemolyticus.* Front. Microbiol. 13: 826151. https://doi.org/10.3389/fmicb.2022.826151

Hoseinifar, S. H., A. Ahmadi, M. Raeisi, S. M. Hoseini, M. Khalili and N. Behnampour (2017). Comparative study on immunomodulatory and growth enhancing effects of three prebiotics (galactooligosaccharide, fructooligosaccharide and inulin) in common carp (*Cyprinus carpio*). Aquac. Res. 48: 3298-3307. https://doi.org/ 10.1111/are.13156.

Ismail, M., A. Wahdan, M. S. Yusuf, E. Metwally and M. Mabrok (2019). Effect of dietary supplementation with a synbiotic (Lacto Forte) on growth performance, hematological and histological profiles, the innate immune response, and resistance to bacterial disease in *Oreochromis niloticus*. Aquac. Res. 50(9): 2545-2562. https://doi.org/10.1111/are.14212.

Kaur, A. P., S. Bahardwaj, D. S. Dhanjal, E. Nepovimona, N. Cruz-Martins, K. Kuca, C. Chopra, R. Singh, H. Kumar, F. Sen, V. Kumar, R. Verma and D. Kumar (2021). Plant prebiotics and their role in the amelioration of diseases. Biomelecules 11(3): 440. https://doi.org/10.3390/ biom11030440.

Kaya, D. (2025). Improvement of brown shrimp (*Penaeus aztecus*) culture parameters through dietary enriched synbiotic in a biofloc system. Aquac. Int. 33: 232. https://doi.org/10.1007/s10499-025-01909-w.

Khanjani, M. H., M. T. Mozanzadeh, E. Gisbert and S. H. Hoseinifa (2024). Probiotics, prebiotics, and synbiotics in shrimp aquaculture: Their effects on growth performance, immune responses, and gut microbiome. Aquac. Rep. 38. https://doi.org/10.1016/j.aqrep.2024.102362.

Knipe, H., B. Temperton, A. Lange, D. Bass and C. R. Tyler (2021). Probiotics and competitive exclusion of pathogens in shrimp aquaculture. Rev. Aquacult. 13: 324-352. https://doi.org/10.1111/raq.12477.

Le Moullac, G., B. Klein, D. Sellos and A. Van Wormhoudt (1997). Adaptation of trypsin, chymotrypsin and a-amylase to casein level and protein source in *Penaeus vannamei* (Crustacea Decapoda). Journal of Experimental Marine Biology and Ecology 208 (1-2): 107-125. https://doi.org/10.1016/S0022-0981(96)02671-8.

Li, Z., M. Liu, Q. Zhou, C. Sun, X. Zheng, J. Chen, B. Liu, S. Liu and A. Wang (2024). Synbiotic effects of *Lactobacillus rhamnosus* fermented with different prebiotics on the digestive enzyme activities, SCFAs and intestinal flora of *Macrobrachium rosenbergii* in vitro. Aquac. Rep. 38. https://doi.org/10.1016/j.aqrep.2024.102303.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall (1951). Protein measurement with the folin phenol reagent. Journal of Biological Chemistry 193(1): 265-275. https//doi:10.1016/s0021-9258(19)52451-6.

Maas, R. M., Y. Deng, Y. Dersjant-Li, J. Petit, M. C. J. Verdegem, J. W. Schrama and F. Kokou (2021). Exogenous enzymes and probiotics alter digestion kinetics, volatile fatty acid content and microbial interactions in the gut of Nile tilapia. Sci. Rep. 11:8221. https://doi.org/10.1038/s41598-021-87408-3.

Mabrouki, S., R. Chalghoumi and H. Abdouli (2017). Effects of pre-germinated fenugreek seeds inclusion in low-fiber diets on post-weaned rabbits' health status, growth performances, carcass characteristics, and meat chemical composition. Trop. Anim. Health Prod. 49: 459-465. https://doi: 10.1007/s11250-016-1214-3.

Mubarokah, F. A., W. Yuliasari and T. S. Wibowo (2023). Phytochemical Screening of Noni (*Morinda citrifolia* L.) leaf ethanol extract in Pejagan Village, Bangkalan Regency. Indonesian Journal of Interdisciplinary Research in Science and Technology 1(7): 661-668. https://doi.org/10.55927/marcopolo.v1i7.5879.

Nababan, Y. I., M. Yuhana, T. Penataseputro, H. Nasrullah, A. Alimuddin and W. Widanarni (2022). Dietary supplementation of *Pseudoalteromonas piscicida* 1UB and fructooligosaccharide enhance growth performance and protect the whiteleg shrimp (*Litopenaeus vannamei*) against WSSV and *Vibrio harveyi* co-infection. Fish Shellfish Immunol. 131: 746-756. https://doi.org/10.1016/j.fsi.2022.10.047.

Nwakanma, C. and G. Akujuobi (2022). Proximate and vitamins assessment of noni juice, leaves and seeds as a functional food security wellness food in global health. Nigeria Journal of Home Economics 9(5): 160-169. https://doi.org/10.61868/njhe.v9i5.30.

Obeng-Boateng, F., S. W. Kpordze and F. Addy (2024). *In vitro* antibacterial activity of *Morinda citrifolia* extracts against eight pathogenic bacteria species. PLoS One 19(10): e0313003. http://doi: 10.1371/journal.pone.0313003.

Oktaviana, A., Widanarni and M. Yuhana (2014). The use of synbiotics to prevent IMNV and *Vibrio harveyi* co-infection in *Litopenaeus vannamei*. Hayati J. Biosci. 21: 127-134, https://doi.org/10.4308/hjb.21.3.127.

Pardede, M. A., Widanarni, Sukenda, M. Yuhana and J. Y. Loh (2024). Effects of dietary synbiotic supplementation on digestive enzyme, total *Vibrio* count, and hepatopancreas of Pacific white shrimp, *Penaeus vannamei* challenged with *Vibrio parahaemolyticus.* Journal of Applied Aquaculture. https://doi.org/10.1080/10454438.2024.2436373.

Pascual, C., G. Gaxiola and C. Rosas (2003). Blood metabolites and hemocyanin of the white shrimp, *Litopenaeus vannamei*: the effect of culture conditions and a comparison with other crustacean species. Marine Biology 142: 735-745. http:// doi.10.1007/s00227-002-0995-2.

Phan, T. C. T., T. K. L. Nguyen, T. T. N. Pham, Q. P. Truong, T. G. Huynh and T. T. H. Tran (2023). Effects of noni fruit extract on the growth performance, digestive enzymes, and stress tolerance of juvenile whiteleg shrimp (*Litopenaeus vannamei*). Egypt. J. Aquat. Res. 49: 549–554. https://doi.org/10.1016/j.ejar.2023.08.003.

Phan, T. C. T., T. T. Nguyen, T. K. L. Nguyen, T. G. Huynh and T. T. H. Tran (2024). Isolation of *Lactobacillus plantarum* CMT1 from shrimp intestine and its effects on growth and survival of the whiteleg shrimp, *Litopeneaus vannamei*. Isr. J. Aquacult. – Bamid. 76(2): 215-222. https://doi.org/10.46989/001c.117555.

Phan, T. C. T, T. K. L. Nguyen, T. T. N. Pham, Q. P. Truong, T. G. Huynh and T. T. H. Tran (2025). Synbiotic effects of *Lactobacillus plantarum* CMT1 and *Morinda citrifolia* on the growth performance and disease resistance of whiteleg shrimp. Comparative Biochemistry and Physiology, Part B 275: 111037. https://doi.org/10.1016/j.cbpb.2024.111037

Prabawati, E., S. Y. Hu, S. T. Chiu, R. Balantyne, Y. Risjani and C. H. Liu (2022). A synbiotic containing prebiotic prepared from a by-product of king oyster mushroom, *Pleurotus eryngii* and probiotic, *Lactobacillus plantarum* incorporated in diet to improve the growth performance and health status of white shrimp, *Litopenaeus vannamei*. Fish Shellfish Immunol. 120: 155-165. http://doi.org./10.1016/j.fsi.2021.11.031.

Rahayu, S., K. Amoah, Y. Huang, J. Cai, B. Wang, V. M. Shija, X. Jin, M. A. Anokyewaa and M. Jiang (2024). Probiotic application in aquaculture: its potential effects, current status in China and future prospects. Frontiers in Marine Science 11: 1455905. https://doi.org/10.3389/fmars.2024.1455905.

Singh, D. R., J. Sunder and R. C. Srivastava (2008). Peptide and mineral profile of *Morinda citrifolia* fruits and leaves. International Journal of Noni Research 2(1–2): 72–78.

Wang, L. U. and J. C. Chen (2005). The immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticu*s at different salinity levels. Fish Shellfish Immunol. 18(4): 269-278. https://doi.org/10.1016/j.fsi.2004.07.008.

Zhang, R., J. Yang, Q. Wang, D. Hu, Q. Zhao, S. Zhu, Y. Qiao, F. Zhao, Z. Wang, J. Wang, Y. Yu, H. Han, L. Hao and H. Dong (2024). Comparative efficacy of plant extracts and probiotics on growth and gut health in chickens with necrotic enteritis. Animals (Basel) 14(22): 3312. http://doi: 10.3390/ani14223312.

Zubaidah, A., M. Yuhana and Widanarni (2015) Encapsulated synbiotic dietary supplementation at different dosages to prevent Vibriosis in white shrimp, *Litopenaeus vannamei*. Hayati J. Biosci. 22: 163-168. https://doi.org/10.1016/j.hjb.2015.10.007.