Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(5): 889 – 907 (2025)
www.ejabf.journals.ekb.eg

Positive Impact of Synbiotic Microcapsules on Immune Response and Microbial Community Modulation in *Litopenaeus vannamei* Challenged with *Vibrio parahaemolyticus*

Abdullah Al Furqon[1], Munti Yuhana[2]*, Widanarni[2], Usamah Afiff[3]

[1]Aquaculture Science, Post Graduate Study Program, Faculty of Fishery and Marine Sciences, IPB University, Darmaga Campus, Bogor, West Java, Indonesia 16680
[2]Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Bogor, Indonesia
[3]Division of Veterinary Disease and Public Health, Veterinary and Biomedical School, IPB University, Bogor, West Java, Indonesia, 16680, Indonesia

***Corresponding Author: muntiyu@apps.ipb.ac.id.**

ARTICLE INFO

Article History:
Received: April 29, 2025
Accepted: Aug. 27, 2025
Online: Sep. 22, 2025

Keywords:
Aquaculture,
Microcapsules,
Synbiotic,
Vibrio parahaemolyticus,
White shrimp

ABSTRACT

This study assessed the protective efficacy of dietary multispecies synbiotic microcapsules, administered at different concentrations, in mitigating *Vibrio parahaemolyticus* infection in white shrimp (*Litopenaeus vannamei*). Juvenile shrimp, averaging 1.54 ± 0.03g in body weight, were randomly distributed into five treatment groups, each with three replicates, including a negative control (K−) and a positive control (K+), and three groups receiving synbiotic supplementation at varying levels (SIN 0.5%, SIN 1%, and SIN 1.5%). Over a 30-day period, the shrimp were given their respective synbiotic-enriched diets five times daily. On the 31st day, all groups, except the negative control, were exposed to *V. parahaemolyticus* at a concentration of 10^6 CFU mL^{-1}. The synbiotic supplement has consisted of mannan oligosaccharides (MOS) as a prebiotic, along with two probiotic strains: *Bacillus cereus* (BR2) and *Pseudoalteromonas piscicida* (1UB). The findings showed that synbiotic treatment significantly (P<0.05) enhanced immune responses, promoted beneficial intestinal colonization by probiotics, reduced *Vibrio parahaemolyticus* levels, and decreased intestinal tissue damage as indicated by histopathological scoring. Overall, the data suggested that dietary supplementation with synbiotic microcapsules improved immune competence and reduced pathogen impact in *Litopenaeus vannamei*, with the 1.5% dosage identified as the most effective.

INTRODUCTION

The white shrimp (*Litopenaeus vannamei*) plays a key role in global aquaculture, with its demand steadily increasing across the world. **FAO (2024)** indicates a notable rise in global shrimp production from 6 million tonnes in 2018 to 7.9 million tonnes by 2022. Indonesia ranks as the fifth-largest shrimp producer. The growing demand has driven the adoption of intensive aquaculture systems which, although capable of producing high yields, frequently subject shrimp to heightened stress levels, suboptimal growth

performance, and a greater vulnerability to disease outbreaks most notably vibriosis caused by *Vibrio parahaemolyticus* **(Abdel-Latif *et al.*, 2022)**.

The presence of pirA and pirB toxin genes in some *Vibrio parahaemolyticus* strains has been connected to AHPND, an extremely deadly disease that may wipe out entire shrimp populations with mortality rates approaching 100% **(Kumar *et al.*, 2016; Sanches-Fernandes *et al.*, 2022)**. AHPND commonly manifests during the initial phase of shrimp cultivation, typically between 15 and 45 days post-stocking **(Peña-Navarro *et al.*, 2020)**. Although antibiotics have historically been employed to manage such infections, their application is now increasingly discouraged due to growing concerns over antimicrobial resistance, along with their detrimental effects on aquatic environments and human health **(Narayanan *et al.*, 2020; Okeke *et al.*, 2022)**.

Synbiotics are increasingly being adopted in aquaculture to improve animal health and promote environmental sustainability **(Hamsah *et al.*, 2019; Yao *et al.*, 2021)**. Probiotics such as *P. piscicida* 1UB and *B. cereus* BR2 have been reported to boost shrimp immunity, reduce infections by *V. parahaemolyticus*, and support improved growth rates **(Nababan *et al.*, 2022; Utomo *et al.*, 2023)**. Prebiotics like mannan oligosaccharides (MOS) have also demonstrated benefits, including improved growth, digestive enzyme activity, and strengthen immune responses in shrimp subjected to pathogenic challenges **(Widanarni *et al.*, 2019; Novriadi *et al.*, 2024)**.

The use of probiotics in live cell form encounters a significant challenge: their limited stability, as they are prone to damage under harsh environmental conditions. To address this issue, microencapsulation methods are used to protect and maintain probiotic viability. Among these, spray drying has proven particularly effective in enhancing probiotic stability and prolonging shelf life **(Ramadhani *et al.*, 2019; Frakolaki *et al.*, 2021)**.

This study seeked to investigate the effectiveness of various doses of synbiotic microcapsules comprising *P. piscicida* 1UB, *B. cereus* BR2, and MOS when incorporated into vaname shrimp feed. The research focused on evaluating their impact on shrimp growth performance, and resistance to vibriosis caused by *V. parahaemolyticus*. Ultimately, these findings are anticipated to support the advancement of more sustainable and environmentally responsible approaches to disease control in *Litopenaeus vannamei* aquaculture.

MATERIALS AND METHODS

Material

The study was conducted using 20 glass aquariums, each with dimensions of 60 × 30 × 35 cm^3 and a capacity of 45 liters of seawater maintained at a salinity of 25 g L^{-1}. To prevent shrimp from escaping, the top of each tank was secured with paranet mesh. The shrimp used in the experiment were specific pathogen-free (SPF), initially weighing an average of 1.54 ± 0.03 g, and each tank housed 20 individual shrimp. This synbiotic blend

included mannan oligosaccharide (MOS) as a prebiotic along with probiotic strains *Bacillus cereus* Tet^R (BR2) and *Pseudoalteromonas piscicida* Tet^R (1UB).

Experimental diets and research design

The test diets were formulated with three different symbiotic supplementation dosages SIN 0.5 (0.5%), SIN 1 (1%), and SIN 1.5 (1.5%) each administered in triplicate. A control group that received no synbiotic supplementation was also included. Two additional control categories were used: a positive control group (K+), which was exposed to *Vibrio parahaemolyticus* Rf^R, and a negative control group (K-), which remained unexposed. For every 1kg of feed, 2.4g of Agribind binder dissolved in 125mL of distilled water was added, followed by the incorporation of synbiotic microcapsules according to the respective treatment dosages. The synbiotic consisted of 0.4% prebiotic MOS and 1% of a probiotic mix (strains 1UB and BR2) at a concentration of 10^7 CFU mL^{-1}. Shrimp were distributed into five groups and fed the respective experimental diets for 30 days. Feeding was scheduled at five intervals daily 06:00 AM, 10:00 AM, 02:00 PM, 06:00 PM, and 10:00 PM. Feeding was initiated at a rate of 8% and subsequently reduced to 6% on day 16 of the culture period.

Preparation of synbiotic microcapsules

The synbiotic microcapsules consisted of whey protein, 10% (w/v) maltodextrin and the main components (probiotics and MOS) and all were combined at a 0.1:1:1 (w/v/v) ratio. To formulate the synbiotic preparation, all ingredients were blended together in a single vessel and stirred continuously for 30 minutes using a magnetic stirrer before undergoing spray drying. The dehydration procedure was performed using a LabPlant SD-Basic spray dryer set to an inlet temperature of 80– 85°C and maintained at an outlet temperature of 50– 55°C, following a modified procedure from **Munaeni *et al.* (2014)**. The microcapsules produced showed a density of 10^7 CFU g^{-1}. Prior to testing, a viability assessment was conducted, and the microcapsules were then stored at an ambient temperature.

Challenge test

On the 31st day, a pathogenicity test was carried out by injecting a suspension of *Vibrio parahaemolyticus* Rf^R cells, which were confirmed to be positive for AHPND. The challenge test was performed through intramuscular injection in the middle body segment, using a 0.1 mL dose of bacterial suspension with a density of 10^5 CFU mL^{-1}. The K- received an equivalent volume of PBS. Mortality rates were recorded daily post-challenge, and any deceased shrimp were swiftly removed from the tanks.

Confirmation of AHPND strain of *Vibrio parahaemolyticus* using PCR

Table 1. PCR-based detection of *Vibrio parahaemolyticus* and Its AHPND-associated genes

Gene	Nucleotide Sequence	Target	Product Length (bp)	Reference
toxR	F: GAA GCA GCA CTC ACC GAT R: GGT GAA GAC TCA TCA GCA	Species-specific identification of *V. parahaemolyticus*	359	(Phiwsaiya *et al.* 2017)
pirA	F: ATG AGT AAC AAT ATA AAA C R: TTA GTG GTA ATA GAT TG	Detection of AHPND toxin gene A	333	(Phiwsaiya *et al.* 2017)
pirB	F: ATG ACT AAC GAA TAC GT R: CTA CTT TTC TGT TAC CAA	Detection of AHPND toxin gene B	1317	(Phiwsaiya *et al.* 2017)
AP4(1)	F: ATG AGT AAC AAT ATA AAA CAT GAA AC R: ACG ATT TCG ACG TTC CCC AA	Confirm AHPND virulence plasmid	1269	(Dangtip *et al.* 2015)
AP4(2)	F: TGA GAA TAC GGG ACG TGG G R: GTT AGT CAT GTG AGC ACC TTC	Confirm AHPND virulence plasmid	230	(Dangtip *et al.* 2015)

Experimental parameters

Immune response

The shrimp underwent anesthesia with clove oil prepared at a 0.3mL L^{-1} concentration. Subsequently, hemolymph samples were collected for analysis. Approximately, 0.2mL of hemolymph was extracted from the first swimming leg base, combined with anticoagulant, and loaded into a hemocytometer for analysis. Hemocyte enumeration was performed under 100x microscopic magnification **(Wang & Chen, 2006)**.

RB activity was measured by assessing superoxide anion (O_2^-) production using NBT. A 100 µL hemolymph-anticoagulant mixture was incubated for 30 minutes, then centrifuged at 3000 rpm for 20 minutes. The supernatant was discarded, and the pellet was stained with 100µL 0.3% NBT solution and incubated for 2 hours. After centrifugation, the pellet was washed with methanol, treated with KOH and DMSO, and measured using a spectrophotometer at 630nm. Respiratory burst was expressed as NBT reduction per 10µL of hemolymph **(Cheng *et al.*, 2004)**.

The PO activity was assessed by mixing 0.2mL of hemolymph with anticoagulant, centrifuging, and resuspending in cacodylate-citrate buffer. The pellet was then treated with trypsin and L DOPA, followed by incubation for 5 minutes. The reaction was terminated by adding additional buffer, and the OD was measured at 492nm using a spectrophotometer **(Liu & Chen, 2004)**.

PA was measured by mixing 100µL of shrimp hemolymph with 25µL of *Staphylococcus aureus* suspension (10^7 CFU mL^{-1}), then incubating for 20 minutes. A smear preparation was made, fixed with methanol for five minutes, and dried. The preparation was then stained with Giemsa stain for 20 minutes. The smear was observed

under a microscope at 400x magnification to assess phagocytic activity, based on the percentage of 100 phagocytic cells showing the phagocytosis process **(Chen *et al.*, 2014)**.

Monitoring of bacterial abundance

Probiotic abundance (1UB Tet^R and BR2 Tet^R), total *vibrio* count (TVC), and total *V. parahaemolyticus* Rf^R count (TVpC) were determined from the intestinal samples. The intestinal organ (fourth abdominal segment) was cut into small pieces using scissors until it was crushed and homogenized. The intestine was then weighed at 0.1g and homogenized again in 0.9mL PBS solution, followed by spreading on agar media. Sea water complete (SWC) agar media, supplemented with tetracycline marker, was used to count probiotic abundance, thiosulphate citrate bile-salt sucrose (TCBS) agar for TVC, and TCBS agar with rifampicin marker for TVpC. The samples were incubated at 28°C, and the number of bacterial colonies that grew was counted after 24 hours, and bacterial counts were determined using the established formula **(Madigan *et al.*, 2012)**.

$$\Sigma \text{ bacterial (CFU mL}^{-1}) = \text{number of colonies x (1/factor dilution) x (1/sampel (mL)}$$

Histopathology

Histopathological analysis of the intestine was observed after the challenge test. Intestinal and hepatopancreas tissues were collected and fixed with Davidson's solution for 24-72 hours. The tissues were then sectioned into 1x1 cm pieces with a thickness of 3-5mm. This was followed by dehydration, clearing, embedding, paraffin blocking, sectioning, and staining. The staining was performed using hematoxylin and eosin (H&E) **(Bell & Lightner, 1988)**. The histopathological evaluation was conducted after the rearing experiments were completed. The prepared slides were examined under an Olympus CX23 light microscope at 40x magnification. Observations were made in five separate fields of view, and the data were analyzed using the formula provided by **Wolf *et al.* (2015)**.

$$P\% = (\Sigma KS / \Sigma TS) \times 100$$

SR = Percentage of necrotic cells
ΣKS = Number of necrotic cells in 5 fields of view
ΣTS = Total number of cells in 5 fields of view

Table 2. Grading the extent of histological damage

Necrosis	Score	Degree of damage
$P < 20\%$	0	Normal
$20\% \leq P < 40\%$	1	Lightly damaged
$40\% \leq P < 60\%$	2	Moderately damaged
$60\% \leq P < 80\%$	3	Severely damaged
$P \leq 80\%$	4	Very heavily damaged

Water quality assessment

Water quality was assessed and daily measurements were taken for temperature and pH, and weekly assessments were conducted for dissolved oxygen (DO) and total ammonia nitrogen (TAN).

Table 3. Water quality measurements during 38 days of rearing

Treatment	TAN	Temperature (°C)	pH	DO (mg L^{-1})
Standard [a]	≤ 1	29-32	7.5-8.5	≥ 4.00
K-	0.07-0.34	27.2-28.4	7.3-7.8	4.33-5.73
K+	0.07-0.35	27.1-28.5	7.2-7.7	4.14-5.71
SIN 0.5	0.08-0.33	27.2-28.4	7.1-7.6	4.11-5.31
SIN 1	0.07-0.36	27.5-28.6	7.3.7.6	4.25-5.28
SIN 1.5	0.07-0.35	27.4-28.3	7.2-7.8	4.26-5.22

Source: Indonesian National Standard [SNI 8008:2014 BSN (2014)].

Data analysis

The data collected during the study were organized and presented in tables using Microsoft Excel 365. Data were tested for normality using the Shapiro-Wilk test, and homogeneity was evaluated using the Levene test. followed by analysis of variance (ANOVA) performed using *IBM SPSS Statistics Ver. 27.* Data that were significantly different were further analyzed using the Tukey test at the 95% confidence interval. Histopathology data were analyzed using scoring.

RESULTS

Immune responses

Health assessments revealed that supplementing shrimp feed with synbiotic microcapsules for 30 days resulted in asignificant increase in health markers compared to the control group. THC reached 13.25 ± 0.45 × 10^6 cells mL^{-1} (Fig. 1a), PA was 44.60 ± 1.71 (Fig. 1b), PO activity was 0.41 ± 0.01 (Fig. 1c), and RB was 0.68 ± 0.15 (Fig. 1d). All these values were significantly greater ($P< 0.05$) compared to the control group (Fig. 1). On days 34 (three days post-challenge) and 38 (seven days post-challenge), shrimp treated with 0.5, 1, and 1.5% SIN doses continued to show significantly higher values ($P< 0.05$). Overall, the 1.5% SIN treatment resulted in enhanced shrimp health, marked by increased THC, PA, RB, and PO levels.

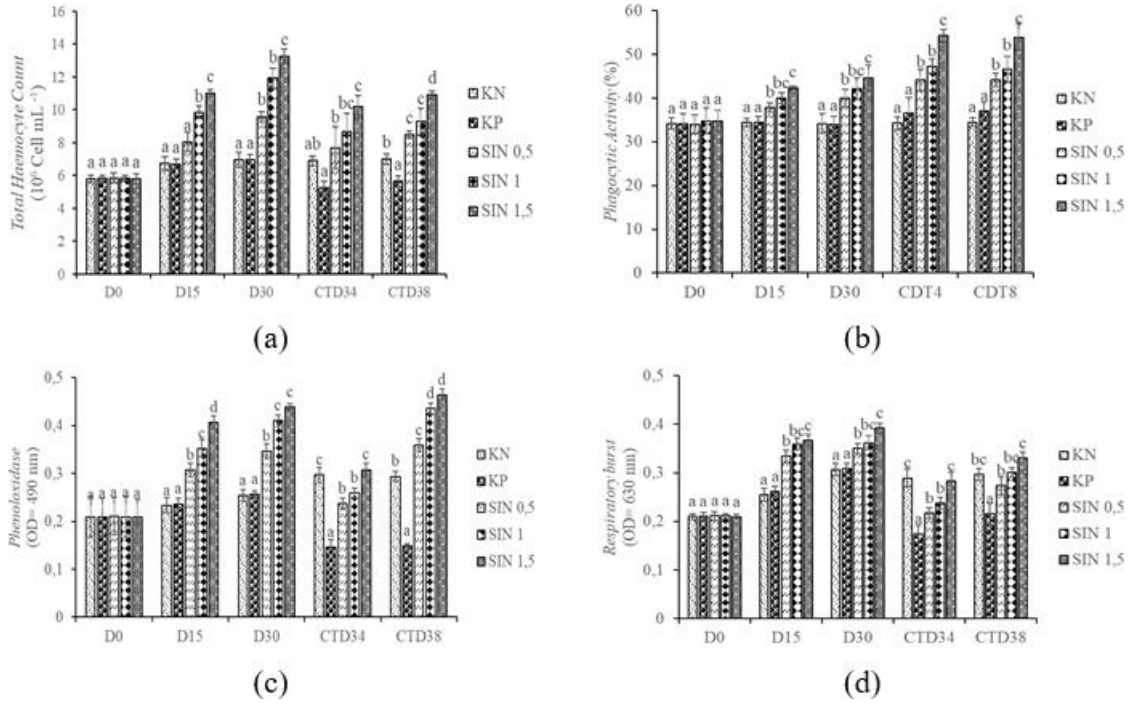


Fig. 1. Total haemocyte count (a), phagocytosis activity (b), phenoloxidase (c), and respiratory burst (d) in white shrimp with varying doses of synbiotic microcapsules. Different superscript letters indicate significant differences at the same observation time, as determined by Tukey's test ($P<0.05$).

Confirmation of *Vibrio parahaemolyticus* carrying toxin using PCR

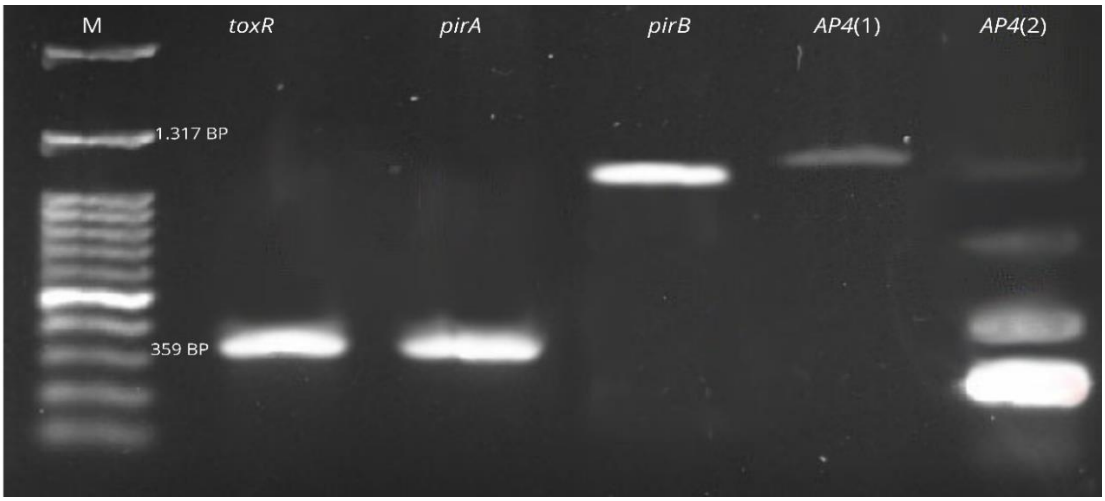
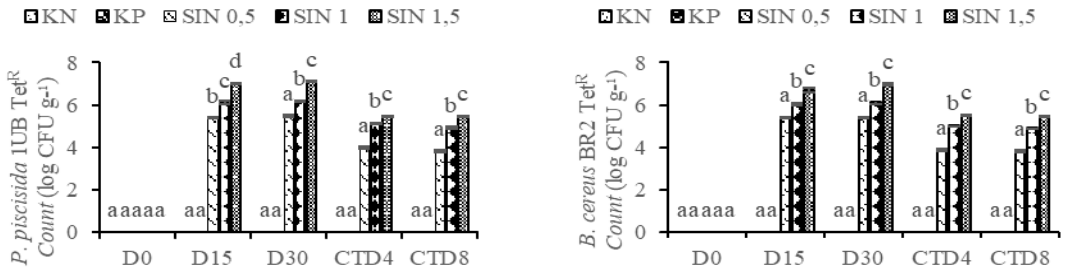


Fig. 2. PCR test of the pathogenic bacterium *Vibrio parahaemolyticus* was performed using specific primers. M: DNA ladder; bands were observed at *toxR* (359 bp), *pirA* (333 bp), *pirB* (1,317 bp), *AP4*(1) (1,269 bp), and *AP4*(2) (230 bp), indicating successful amplification of the target genes.

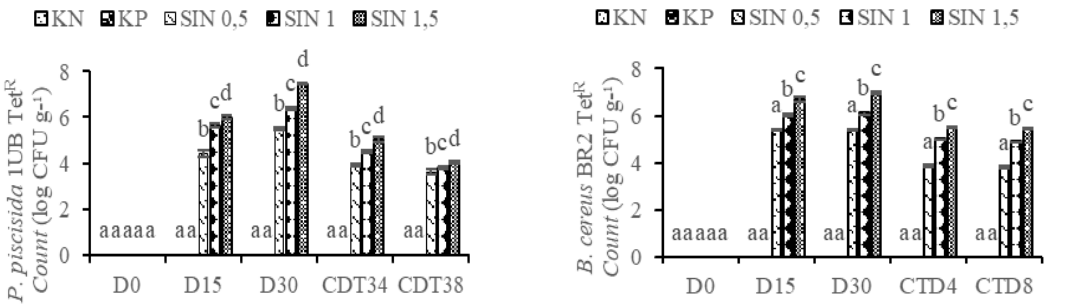
Bacterial abundance monitoring

The supplementation of synbiotic microcapsules over 30 days effectively reduced the total *Vibrio* species count (TVC) ($P<0.05$) while boosting the abundance of probiotic bacteria (*Pseudoalteromonas piscicida* 1UB Tet^R and *Bacillus cereus* BR2 Tet^R) in the hepatopancreas and intestines of white shrimp. This was observed in comparison to both positive and negative controls before the challenge test (Figs. 3, 4, 5, and 6). The TVC and total *Vibrio parahaemolyticus* Rf^R count (TVpC) values (Fig. 7) showed an increase on day 34 (three days post-challenge), followed by a decrease on day 38 (seven days post-challenge). Treatments with SIN 1 and SIN 1.5 exhibited significantly lower *Vibrio* populations than the positive control ($P< 0.05$). In general, the 1.5% SIN treatment resulted in the most notable reduction in TVC and TVpC values ($P<0.05$).



(a) (b)

Fig. 3. Total abundance of probiotic bacteria: *P. piscicide* 1UB Tet^R (a) and *B. cereus* BR2 Tet^R (b) in the hepatopankreas organs of white shrimp during 30 days rearing and 7 days challenge test. Different superscript letters indicate significant differences at the same observation time, as determined by Tukey's test ($P<0.05$).



(a) (b)

Fig. 4. Total abundance of probiotic bacteria: *P. piscicide* 1UB Tet^R (a) and *B. cereus* BR2 Tet^R (b) in the intestine of white shrimp during 30 days (day of rearing/D) and post challenge test (CTD4 and CTD8). Different superscript letters indicate significant differences at the same observation time, as determined by Tukey's test ($P<0.05$).

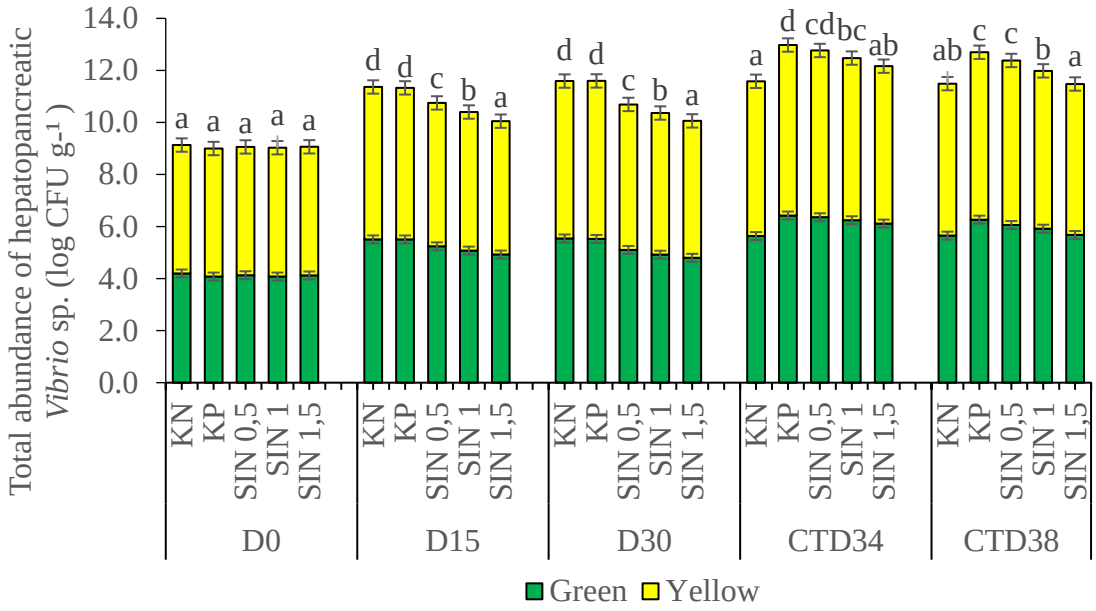


Fig. 5. Total abundance of hepatopancreatic *Vibrio* sp.: *yellow colonies* and *green colonies* on TCBS agar medium during 30 days of rearing and 7 days of challenge test. Different superscript letters indicate significant differences at the same observation time, as determined by Tukey's test (P<0.05).

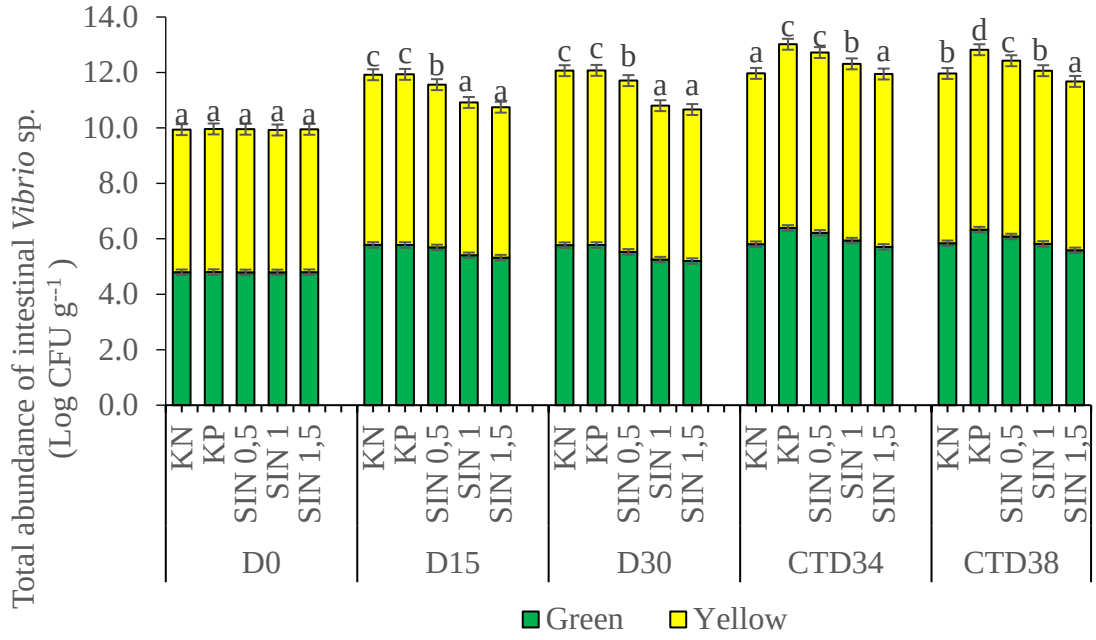
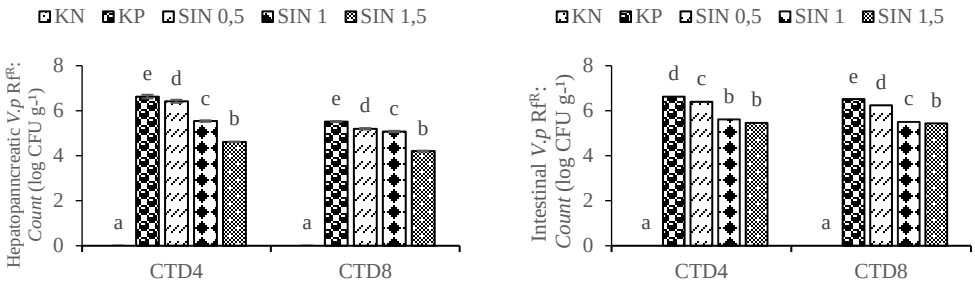


Fig. 6. Total abundance of intestinal *Vibrio* sp.: *yellow colonies* and *green colonies* on TCBS agar medium during 30 days of rearing and 7 days of challenge test. Different superscript letters indicate significant differences at the same observation time, as determined by Tukey's test (P<0.05).



(a) (b)

Fig. 7. Total *Vibrio parahaemolyticus* Rf^R bacteria in hepatopancreas (a) and *Vibrio parahaemolyticus* Rf^R in intestine (b) of white shrimp on day 7^{th} post challenge test. Different superscript letters indicate significant differences at the same observation time, as determined by Tukey's test (P<0.05).

Histopathology

Histopathological assessment of the intestinal tissue from the white shrimp was conducted on day 38, which corresponds to seven days following the challenge test (Fig. 8). The observations revealed that the intestinal morphology in the negative control group (K-) remained intact. In contrast, shrimp treated with 0.5, 1, and 1.5% SIN showed significantly less tissue disruption than those in the positive control group (P< 0.05). The histopathological evaluation also indicated that infection with *Vibrio parahaemolyticus* caused varying degrees of intestinal damage, ranging from mild to severe. Nonetheless, the severity of intestinal damage was markedly reduced (P< 0.05) in shrimp receiving synbiotic microcapsules when compared with the positive control group. Further tissue damage classification revealed that the K- group displayed normal tissue architecture, all synbiotic-fed groups exhibited only mild alterations, whereas the positive control group experienced extensive tissue damage (Table 4).

Table 4. Assessment and extent of histopathological damage in the intestine of white shrimp after *V. parahaemolyticus* challenge test

Treatment	Necrosis (%)	Score	Degree of Damage
K-	17,41%	0	Normal
K+	61,35%	3	Severely damaged
SIN 0.5	31,73%	2	Mildly damaged
SIN 1	24,68%	2	Mildly damaged
SIN 1.5	21,82%	2	Mildly damaged

Note: Histopathological damage was categorized based on the percentage of affected tissue as follows less than 20% (score 0, normal), 20–39% (score 1, mild), 40–59% (score 2, moderate), 60-79% (score 3, severe), and 80% or more (score 4, very severe).

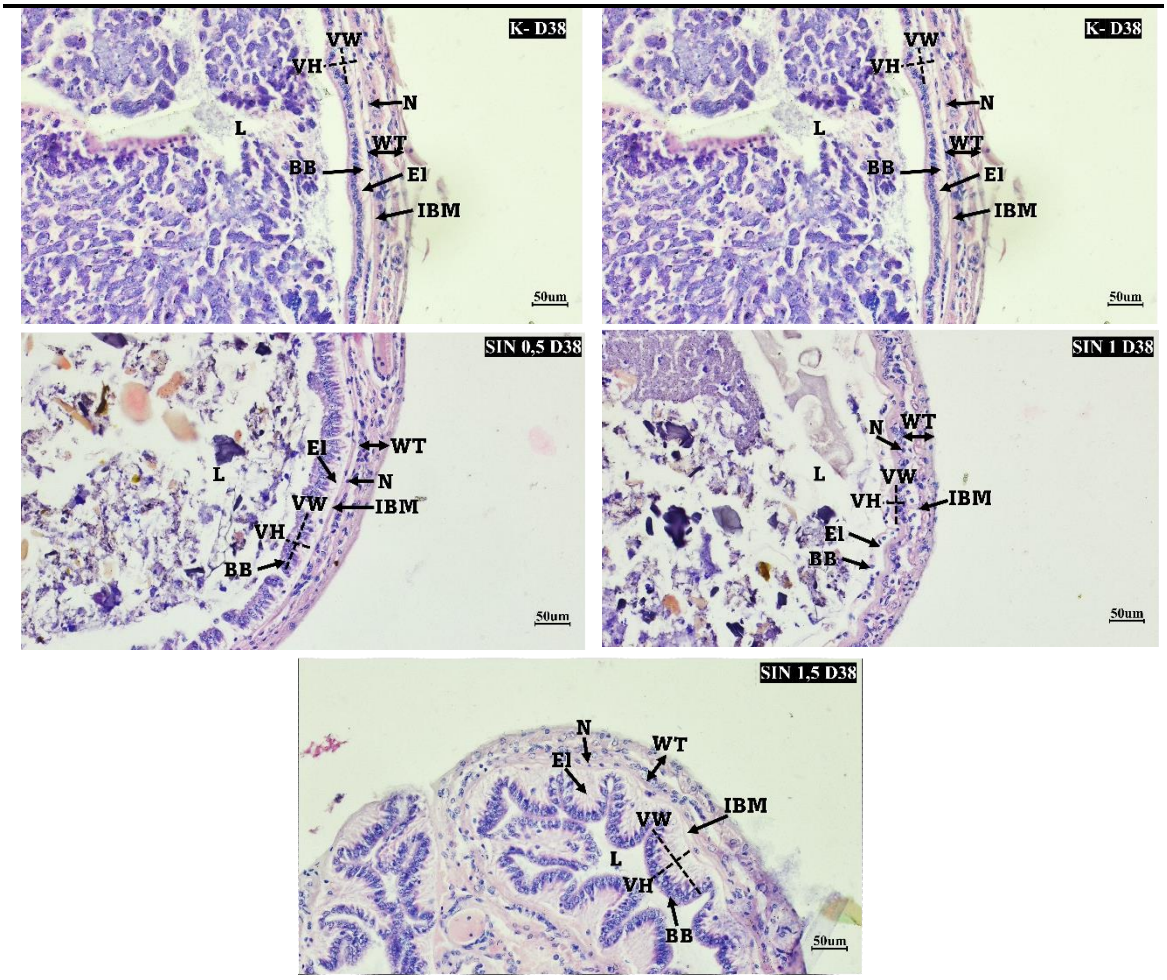


Fig. 8. Histopathological examination of the Pacific white shrimp's intestine at the end of the rearing period. N, nuclei; IBM, irregular basement membrane; VH, villus height; VW, villus weight; L, lumen; EI, epithelium intestine; WT, wall thickness; BB, brush border.

DISCUSSION

The health condition of white shrimp can be assessed through their haemocyte profiles. Haemocytes are involved in identifying and neutralizing invading pathogens, and they mediate immune responses through several mechanisms, such as phagocytosis, nodule formation, melanization, cell to cell signaling, and cytotoxic activities, which vary depending on the characteristics of the pathogen **(Eleftherianos *et al.*, 2021)**. In this study, dietary supplementation with 1.5% synbiotic (SIN) microcapsules was found to be the most effective dose in boosting immune parameters (Fig. 1).

The enhanced THC values indicate that the synbiotic microcapsules may act as immunostimulants, encouraging haemocyte proliferation. This immune-enhancing effect is likely due to the combined action of probiotics and prebiotics, which influence the host's immune system through pattern recognition receptors (PRRs) that recognize microbial

signatures known as pathogen-associated molecular patterns (PAMPs). These PAMPs consist of structural elements like lipopolysaccharides (LPS) from Gram-negative bacteria, peptidoglycan (PGN) and lipoteichoic acid (LTA) from Gram-positive bacteria, along with glucans from yeast and microbial polynucleotides **(Wang & Wang, 2013; Huynh *et al.*, 2017)**. Moreover, the inclusion of MOS in the microcapsules is likely to contribute to immune modulation, potentially via the lectin binding pathway **(Torrecillas *et al.*, 2014)**.

When infected with *Vibrio parahaemolyticus*, shrimp typically show reduced levels of THC, PO, and RB, which is attributed to the limited phagocytic capability against the pathogen **(Havanapan *et al.*, 2016)**. During infection, haemocytes migrate toward the site of infection, leading to a drop in haemocyte concentration in the haemolymph. These cells tend to cluster and encapsulate the pathogen at the injection site. Furthermore, apoptosis in haematopoietic tissues also contributes to the decline in haemocyte numbers post-infection, which correlates with more severe infections and a higher risk of shrimp mortality **(Yeh *et al.*, 2009)**.

The evaluation of phagocytic activity (PA) showed a significant enhancement in the SIN 1.5 group relative to the other treatments. This immune response entails haemocytes engulfing invading particles, representing a vital mechanism in the shrimp's defense against pathogenic threats **(Liu *et al.*, 2020)**. The enhanced PA suggests that administering synbiotic microcapsules through feed effectively boosts the shrimp's immune response. This is consistent with the mode of action of immunostimulants, which enhance immune responses by stimulating the PA cells **(Kumar *et al.*, 2023)**. In addition, the rise in total haemocyte count (THC) and phagositosis activity (PA) reflects a robust primary defense mechanism against infections **(Panigrahi *et al.*, 2020)**.

Respiratory burst (RB), a component of the shrimp's innate immunity, is activated during pathogen invasion. This mechanism takes place as haemocytes internalize pathogens, forming phagosomes that subsequently release enzymes to produce reactive oxygen intermediates (ROIs), which help neutralize the invading microbes **(Risjani *et al.*, 2021)**. In this experiment, shrimp that received a synbiotic microcapsule diet for 30 days exhibited elevated RB levels compared to the control group. Haemocytes are thought to contribute significantly to elevated ROI levels when responding to pathogenic challenges. The observed reduction in RB levels three days after the challenge may be linked to the movement of haemocytes toward infection sites, where they engage in phagocytic activity and trigger immune responses **(Muharrama *et al.*, 2022)**.

Phenoloxidase (PO) activity was notably increased in the SIN 1 and SIN 1.5 treatment groups, both prior to and following exposure to the pathogen. This enzyme is part of the humoral immune defense, initially stored in an inactive prophenoloxidase (proPO) form within the haemolymph **(Mahasri *et al.*, 2018; Barrios *et al.*, 2023)**. High PO activity demonstrates the shrimp's ability to detect and react to pathogens, signaling a heightened immune defense. The PO cascade begins with phenol oxidation into quinones,

which subsequently form melanin pigments that immobilize and neutralize pathogens through melanisation **(Zhao *et al.*, 2011)**.

The synbiotic microcapsules were effective in combating vibriosis, evidenced by increased populations of beneficial bacteria (1UB Tet^R and BR2 Tet^R) over 30 days, and reductions in total *Vibrio* counts (TVC) and total *Vibrio parahaemolyticus* counts (TVpC) after the challenge test (Figs. 3–7). This bacterial proliferation suggests the probiotics successfully colonized the shrimp's intestine, hindering pathogen attachment. According to **Xie *et al*. (2019)**, *Lactobacillus* supplementation enhances intestinal bacterial diversity without harmful effects. The prebiotic component, MOS, further supports intestinal health, boosts microbiota diversity, improves nutrient absorption, and promotes shrimp growth **(Gainza & Romero, 2020)**.

Probiotic supplementation led to a higher presence of beneficial microbes while suppressing *Vibrio parahaemolyticus* Rf^R levels relative to the positive control group. The presence of 1UB Tet^R and BR2 Tet^R strains appears to outcompete the pathogen, sustaining intestinal colonization post challenge test period even without additional microcapsule feeding (Figs. 3, 4). Additionally, *P. piscicida*, a Gram-negative bacterium, is known to produce antibacterial and antibiofilm substances that degrade pathogen proteins **(Wang *et al.*, 2018)**. It can attach to *V. parahaemolyticus*, deliver lytic vesicles, and cause cell wall damage that ultimately destroys infected cells **(Richards *et al.*, 2017)**.

Bacillus cereus, whether live or inactivated (as paraprobiotics), has been shown to positively influence shrimp stress resistance, immunity, and digestive enzyme performance **(Yuhana *et al.*, 2021, 2024)**. These benefits likely stem from both metabolite production and bacterial cell wall interactions with the host's intestinal lining. This suggests that synbiotic microcapsules not only enhance shrimp health but also contribute to aquaculture productivity. *Bacillus* species naturally serve as antimicrobial producers, supporting immune processes and environmental regulation in the intestine **(Yuhana *et al.*, 2022)**. When combined with prebiotics, they demonstrate a synergistic effect against pathogenic microbes, enhancing the shrimp's immune strength **(Mohan *et al.*, 2019)**.

Histological analysis at the end of the rearing period showed that shrimp receiving synbiotic microcapsules experienced less intestinal damage following pathogen challenge, in contrast to the positive control group (Fig. 8). In contrast, the negative control group maintained normal intestinal architecture, with intact villi and clear structure. Severe intestinal damage in the positive control group (scoring 61.35%) included disrupted epithelial structure, loss of villus boundaries, and necrotic cells (Table 3). Synbiotic supplementation preserved intestinal morphology, inhibited pathogenic bacteria, and maintained tissue integrity. These results align with past findings indicating that synbiotics support the intestinal barrier, with orderly columnar epithelium, clear luminal borders, and intact villi structure **(Pardede *et al.*, 2023; Ermawati *et al.*, 2024)**.

CONCLUSION

The incorporation of synbiotic microcapsules into shrimp diets effectively enhanced immune performance and reduced the density of *Vibrio parahaemolyticus*. The most pronounced outcomes were recorded at a 1.5% supplementation level.

ETHICS APPROVAL

All experimental procedures involving fish in this research adhered to established animal welfare standards, based on protocol number 279-2024. Ethical approval was granted by the Ethics Committee on Animal Use of IPB University in October 2024.

ACKNOWLEDGMENTS

We extend our sincere appreciation to the Indonesian Ministry of Higher Education, Science, and Technology for funding this study through the Fundamental Research Scheme (Grant No. 436/IT3.D10/PT.01.03/P/B/2024), awarded to Dr. Munti Yuhana.

REFERENCES

Abdel-Latif, HMR.; Yilmaz, E.; Dawood, MAO.; Ringø, E.; Ahmadifar, E. and Yilmaz, S. (2022). Shrimp vibriosis and possible control measures using probiotics, postbiotics, prebiotics, and synbiotics: A review. *Aquaculture*, 551. https://doi.org/10.1016/j.aquaculture.2022.737951

[BSN] Badan Standardisasi Nasional. (2014). Intensive white shrimp (*Litopenaeus vannamei*. Boone, 1931) production in lining ponds SNI 8008:2014. Jakarta: Badan Standardisasi Nasional.

Barrios, Y.; Roncarati, A.; Martín, RLD.; Rodríguez, GM.; González-Salotén, M.; López-Zaldívar, Y. and Arenal, A. (2023). Effects of fructooligosaccharides (fos) on the immune response of the shrimp *Penaeus vannamei* and on the reduction in *Vibrio* spp. and *Pseudomonas* spp. in cultures of post-larvae. *Microbiology Research*, 14(3): 870–882. https://doi.org/10.3390/microbiolres14030060

Bell, TA. and Lightner, DV. (1988). A handbook of normal penaeid shrimp histology. Baston: World Aquaculture Society.

Chen, YY.; Chen, JC.; Lin, YC.; Putra, DF.; Kitikiew, S.; Li, CC.; Hsieh, JF.; Lious, CH. and Yeh, ST. (2014). Shrimp that have received carrageenan via immersion and diet exhibit immunocompetence in phagocytosis despite a post-plateau in immune parameters. *Fish Shellfish Immunol.*, 36: 352-366. https://doi.org/10.1016/j.fsi.2013.12.004

Cheng, W.; Hsiao, IS.; Hsu, CH. and Chen, JC. (2004). Change in water temperature on the immune response of Taiwan abalone *Haliotis diversicolor* supertexta and its

susceptibility to *Vibrio parahaemolyticus*. *Fish Shellfish Immunol*., 17(3): 235–243. https://doi.org/10.1016/j.fsi.2004.03.007

Dangtip, S.; Sirikharin, R.; Sanguanrut, P.; Thitamadee, S.; Sritunyalucksana, K.; Taengchaiyaphum, S.; Mavichak, R.; Proespraiwong, P. and Flegel ,TW. (2015). AP4 method for two tube nested PCR detection of AHPND isolates of *Vibrio parahaemolyticus*. *Aquaculture Reports*, 2: 158-162. https://doi.org/10.1016/j.aqrep.2015.10.002

Eleftherianos, I.; Heryanto, C.; Bassal, T.; Zhang, W.; Tettamanti, G. and Mohamed, A. (2021). Haemocyte-mediated immunity in insects: Cells, processes and associated components in the fight against pathogens and parasites. *Immunology*, 164(3): 401–432. https://doi.org/10.1111/imm.13390

Ermawati, RP.; Yuhana, M.; Widanarni, W. and Afiff, U. (2024). Field testing of dual species synbiotic microcapsules during IMNV disease outbreak and fluctuate weather: growth performance and immune response in Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture International*, 32: 4529–4550 https://doi.org/10.1007/s10499-024-01389-4

[FAO] Food and agriculture organisation of the united nations. (2024). The state of world fisheries and aquaculture 2024. Rome. www.fao.org/66538eba-9c85-4504-8438c1cf0a0a3903/content/cd0683en.html

Frakolaki, G.; Giannou, V.; Kekos, D. and Tzia, C. (2021). A review of the microencapsulation techniques for the incorporation of probiotic bacteria in functional foods. *Crit Rev Food Sci Nutr*., 61(9):1515–1536. https://doi.org/10.1080/10408398.2020.1761773

Gainza, O. and Romero, J. (2020). Effect of mannan oligosaccharides on the microbiota and productivity parameters of *Litopenaeus vannamei* shrimp under intensive cultivation in Ecuador. *Sci Rep*., 10(1). https://doi.org/10.1038/s41598-020-59587-y

Hamsah, H.; Widanarni, W.; Alimuddin, A.; Yuhana, M.; Junior, MZ. and Hidayatullah, D. (2019). Immune response and resistance of Pacific white shrimp larvae administered probiotic, prebiotic, and synbiotic through the bio-encapsulation of *Artemia* sp. *Aquaculture International*, 27(2):567–580. https://doi.org/10.1007/s10499-019-00346-w

Havanapan, PO.; Taengchaiyaphum, S.; Ketterman, AJ. and Krittanai, C. (2016). Yellow head virus infection in black tiger shrimp reveals specific interaction with granule-containing hemocytes and crustinPm1 as a responsive protein. *Developmental & Comparative Immunology*, 54(1): 126-136. https://doi.org/10.1016/j.dci.2015.09.005

Huynh, TG.; Shiu, YL.; Nguyen, TP.; Truong, QP.; Chen, JC. and Liu, CH. (2017). Current applications, selection, and possible mechanisms of actions of synbiotics in improving the growth and health status in aquaculture: A review. *Fish Shellfish Immunol*., 64:367–382. https://doi.org/10.1016/j.fsi.2017.03.035

Kumar, S.; Verma, AK.; Singh, SP. and Awasthi, A. (2023). Immunostimulants for shrimp aquaculture: paving pathway towards shrimp sustainability. *Environmental Science and Pollution Research*, 30(10) :25325–25343. https://doi.org/10.1007/s11356-021-18433-y

Kumar, V.; Roy S.; Meena, DK. and Sarkar, UK. (2016). Application of probiotics in shrimp aquaculture: importance, mechanisms of action, and methods of administration. *Reviews in Fisheries Science and Aquaculture*, 24(4): 342–368. https://doi.org/10.1080/23308249.2016.1193841

Liu, CH. and Chen, JC. (2004). Effect of ammonia on the immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus*. *Fish Shellfish Immunology*, 16: 321–334. https://doi.org/10.1016/S1050-4648(03)00113-X

Liu, F.; Li, S.; Yu, Y.; Sun, M.; Xiang, J. and Li, F. (2020). Effects of ammonia stress on the hemocytes of the Pacific white shrimp *Litopenaeus vannamei*. *Chemosphere*, 239. https://doi.org/10.1016/j.chemosphere.2019.124759

Madigan, MT.; Martinko, JM.; Stahl, DA. and Clark, DP. (2012). Brock Biology of Microorganisms, 13th edn. Benjamin Cummings, San Francisco (US)

Mahasri, G.; Kusdarwati, R.; Kismiyati.; Rozi. and Gustrifandi, H. (2018). Effectivity of immunostimulant from *Zoothamnium penaei* protein membrane for decreasing the mortality rate of white shrimp (*Litopenaeus vannamei*) in traditional plus pond. *IOP Conference Series: Earth and Environmental Science*, 137. https://doi.org/10.1088/1755-1315/137/1/012020

Mohan, K.; Ravichandran, S.; Muralisankar, T.; Uthayakumar, V.; Chandirasekar, R.; Seedevi, P. and Rajan, D. (2018). Potential uses of fungal polysaccharides as immunostimulants in fish and shrimp aquaculture: a review. *Aquaculture*, 500: 250–263. https://doi.org/10.1016/j.aquaculture.2018.10.023

Muharrama, ARW.; Widanarni, W.; Alimuddin, A. and Yuhana, M. (2022). Gene expression and immune response of pacific white shrimp given *Bacillus* NP5 probiotic and honey prebiotic and *Vibrio parahaemolyticus* infection. *Journal of Applied Aquaculture*, 34(3): 625–641. https://doi.org/10.1080/10454438.2021. 1873888

Munaeni, W.; Yuhana, M. and Widanarni, W. (2014). Effect of Microencapsulated Synbiotic at Different Frequencies for Luminous *Vibriosis* Control inWhite Shrimp (*Litopenaeus vannamei*). *Microbiol Indones*., 8(2):73–80. https://doi.org/10.5454/mi.8.2.5

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

Narayanan, SV.; Joseph, TC.; Peeralil, S.; Koombankallil, R.; Vaiyapuri, M.; Mothadaka, MP. and Lalitha KV. (2020). Tropical shrimp aquaculture farms

harbour pathogenic *Vibrio parahaemolyticus* with high genetic diversity and Carbapenam resistance. *Mar Pollut Bull*., 160: 111551 https://doi.org/10.1016/j.marpolbul.2020.111551

Novriadi, R.; Fayuan G.; Davies, S.; Istiqomah, I.; Isnansetyo, A.; Farkan, M.; Jinjun, D.; Jianhua, Y.; Xin, H. and Yan, Z. (2024). Evaluation of dietary yeast derived mannan oligosaccharide for pacific white shrimp *Penaeus vannamei*: Effects on growth performance, immune response, hepatopancreas morphology and resilience to infection against *Vibrio parahaemolyticus*. *Aquac Rep*., 38. https://doi.org/10.1016/j.aqrep.2024.102307

Okeke, ES.; Chukwudozie, KI.; Nyaruaba, R.; Ita, RE.; Oladipo, A.; Ejeromedoghene, O.; Atakpa, EO.; Agu, CV. and Okoye, CO. (2022). Antibiotic resistance in aquaculture and aquatic organisms: a review of current nanotechnology applications for sustainable management. *Environmental Science and Pollution Research*, 29(46): 69241–69274. https://doi.org/10.1007/s11356-022-22319-y

Panigrahi, A.; Saranya, C.; Ambiganandam, K.; Sundaram, M.; Sivakumar, MR. and Kumaraguru, VKP. (2020). Evaluation of biofloc generation protocols to adopt high density nursery rearing of *Penaeus vannamei* for better growth performances, protective responses and immuno modulation in biofloc based technology. *Aquaculture*, 522. https://doi.org/10.1016/j.aquaculture.2020.735095

Pardede, MA.; Widanarni, W.; Sukenda,, S.; and Yuhana, M. (2023). Evaluation of dietary microencapsulated synbiotic *Pseudoalteromonas piscicida* 1UB, *Bacillus* NP5, and mannan-oligosaccharides to prevent *Vibrio parahaemolyticus* infection in Pacific white shrimp *Penaeus vannamei*. *Aquaculture International*, 32, 2077–2091. https://doi.org/10.1007/s10499-023-01258-6

Peña-Navarro, N.; Castro-Vásquez, R.; Vargas-Leitón, B.; and Dolz, G. (2020). Molecular detection of acute hepatopancreatic necrosis disease (AHPND) in *Penaeus vannamei* shrimps in Costa Rica. *Aquaculture*, 523. https://doi.org/10.1016/j.aquaculture.2020.735190

Phiwsaiya, K.; Charoensapsri, W.; Taengphu, S.; Dong, HT.; Sangsuriya, P.; Nguyen, GTT.; Pham, HQ.; Amparyup, P.; Sritunyalucksana, K. and Taengchaiyaphum, S. (2017). A natural *Vibrio parahaemolyticus pirA* vp *pirB* vp mutant kills shrimp but produces neither pir vp toxins nor acute hepatopancreatic necrosis disease lesions. *Environ Microbiol.*, (16). https://doi.org/0.1128/AEM.00680-17

Ramadhani, DE.; Widanarni, W. and Sukenda, S. (2019). Microencapsulation of probiotics and its applications with prebiotic in Pacific white shrimp larvae through *Artemia* sp. *Jurnal Akuakultur Indonesia*, 18(2): 130–140. https://doi.org/10.19027/jai.18.2.130-140

Richards, GP.; Watson, MA.; Needleman, DS.; Uknalis, J.; Boyd, EF. and Fay, JP. (2017). Mechanisms for *Pseudoalteromonas piscicida* induced killing of *vibrios* and

other bacterial pathogens. *Appl Environ Microbiol*., 83(11). https://doi.org/10.1128/AEM.00175-17

Risjani Y.; Mutmainnah N.; Manurung P.; Wulan SN. and Yunianta. (2021). Exopolysaccharide from *Porphyridium cruentum* (*purpureum*) is not toxic and stimulates immune response against *Vibriosis*: the assessment using zebrafish and white shrimp *Litopenaeus vannamei*. *Mar Drugs*, 19(3). https://doi.org/10.3390/MD19030133

Sanches-Fernandes, GMM.; Sá-Correia, I. and Costa, R. (2022). Vibriosis outbreaks in aquaculture: addressing environmental and public health concerns and preventive therapies using gilthead seabream farming as a model system. *Front Microbiol*., 13 https://doi.org/10.3389/fmicb.2022.904815

Torrecillas, S.; Montero, D. and Izquierdo, M. (2014). Improved health and growth of fish fed mannan oligosaccharides: Potential mode of action. *Fish Shellfish Immunol*., 36(2): 525–544. https://doi.org/0.1016/j.fsi.2013.12.029

Utomo, AS.; Yuhana, M.; Widanarni, W. and Afiff, U. (2023). Immune response, gene expression, and intestinal microbial composition of Pacific white shrimp fed with multispecies synbiotic for the prevention of coinfection disease. *Aquaculture International*, 31(1): 53–64. https://doi.org/10.1007/s10499-022-00966-9

Wang, FI. and Chen, JC. (2006). Effect of salinity on the immune response of tiger shrimp *Penaeus monodon* and its susceptibility to *Photobacterium damselae* subsp. *damselae. Fish Shellfish Immunol.*, 20: 671–681. https://doi.org/10.1016/j.fsi.2005.08.003

Wang, H.; Wang, C.; Tang, Y.; Sun, B.; Huang, J. and Song, X. (2018). *Pseudoalteromonas* probiotics as potential biocontrol agents improve the survival of *Penaeus vannamei* challenged with acute hepatopancreatic necrosis disease (AHPND) causing *Vibrio parahaemolyticus. Aquaculture*, 494: 30-36. https://doi.org/10.1016/j.aquaculture.2018.05.020

Wang, XW. and Wang, JX. (2013). Pattern recognition receptors acting in innate immune system of shrimp against pathogen infections. *Fish Shellfish Immunol.*, 34(4: 981-989. https://doi.org/10.1016/j.fsi.2012.08.008

Widanarni, W.; Taufik, A.; Yuhana, M. and Ekasari, J. (2019). Dietary mannan oligosaccharides positively affect the growth, digestive enzyme activity, immunity and resistance against *Vibrio harveyi* of pacific white shrimp (*Litopenaeus vannamei*) larvae. *Turk J Fish Aquat Sci*., 19(4). https://doi.org/10.4194/1303-2712-v19_4_01

Wolf, JC.; Baumgartner, WA.; Blazer, VS.; Camus, AC.; Engelhardt, JA.; Fournie, JW.; Frasca, S.; Groman, DB.; Kent, ML. and Khoo, LH. (2015). Nonlesions, misdiagnoses, missed diagnoses, and other interpretive challenges in fish histopathology studies: A guide for investigators, authors, reviewers, and readers. *Toxicol Pathol*., 43(3):297–325. https://doi.org/10.1177/0192623314540229

Xie, JJ.; Liu, QQ.; Liao, S.; Fang, HH.; Yin, P.; Xie, SW. and Niu, J. (2019). Effects of dietary mixed probiotics on growth, non-specific immunity, intestinal morphology and microbiota of juvenile pacific white shrimp, *Litopenaeus vannamei*. *Fish Shellfish Immunol.*, 90, 456–465. https://doi.org/10.1016/j.fsi.2019.04.301.

Yao, W.; Li, X.; Zhang, C.; Wang, J.; Cai, Y. and Leng X. (2021). Effects of dietary synbiotics supplementation methods on growth, intestinal health, non-specific immunity and disease resistance of Pacific white shrimp, *Litopenaeus vannamei. Fish Shellfish Immunol*., 112:46–55. https://doi.org/10.1016/j.fsi.2021.02.011

Yeh, SP.; Chen, YN.; Hsieh, SL.; Cheng, W. and Liu, CH. (2009). Immune response of white shrimp, *Litopenaeus vannamei*, after a concurrent infection with white spot syndrome virus and infectious hypodermal and hematopoietic necrosis virus. *Fish & Shellfish Immunology*, 26(4): 582-588. https://doi.org/10.1016/j.fsi.2008.09.010

Yuhana, M.; Ramadhan, DA.; Krettiawan, H. and Afiff, U. (2021). Growth performance and stress resistance on early stage of *Macrobrachium rosenbergii* fed with *Bacillus* sp. supplementation. *Jurnal Teknologi Perikanan dan Kelautan*, 12(1): 85–92. https://doi.org/10.24319/jtpk.12.85-92

Yuhana, M.; Tambun, A.; Widanarni, W. and Afiff, U. (2022). Synbiotic microcapsule dietary supplementation for prevention against co-infection diseases in Pacific white shrimp: a limited field experiment. *Jurnal Akuakultur Indonesia*, 21(2): 125-132. https://doi.org/10.19027/jai.21.2.125-132

Yuhana, M.; Asvia, S.; Widanarni, W. and Afiff, U. (2024). Improvement in growth, enzyme activity, and gene expression against *Edwardsiellosis* in African catfish (*Clarias* sp.) with dietary supplementation of euryhaline probiotic and paraprobiotic *Bacillus cereus* BR2. *Aquaculture International*, 32(7): 9303–9319. https://doi.org/10.1007/s10499-024-01617-x

Zhao, P.; Lu, Z.; Strand, MR. and Jiang, H. (2011). Antiviral, anti-parasitic, and cytotoxic effects of 5,6-dihydroxyindole (DHI), a reactive compound generated by phenoloxidase during insect immune response. *Insect Biochem Mol Biol*., 41(9):645–652. https://doi.org/10.1016/j.ibmb.2011.04.006