JPUGM
JURNAL PERIKANAN UNIVERSITAS GADJAH MADA
International Journal in Aquatic and Fisheries Sciences
ISSN: 2502-5066 (Online)
ISSN: 0853-6384 (Print)
Available online at
https://jurnal.ugm.ac.id/jfs

Field-Scale Application of Multispecies Probiotics as A Sustainable Strategy to Boost Health and Productivity of Pacific White Shrimp (*Penaeus vannamei*)

Lindah Erlia Safira Siregar[1], Sinung Rahardjo[2*], Tb. Haeru Rahayu[3] & Putu Angga Wiradana[4]
[1]Aquaculture Technology Study Program, Applied Bachelor Program, Jakarta Technical University of Fisheries, Jakarta, Indonesia
[2]Study Program of Fisheries Resources Utilization, Jakarta Technical University of Fisheries, Jakarta, Indonesia
[3]Directorate General of Aquaculture, Ministry of Marine Affairs and Fisheries (MMAF/KKP), Jakarta, Indonesia
[4]Study Program of Biology, Faculty of Health and Science, Universitas Dhyana Pura, Badung, Bali, Indonesia
*Corresponding author, email: snngrahardjo@gmail.com

OPEN ACCESS

Article Info

Submitted	: 10 October 2025
Revised	: 10 December 2025
Accepted	: 15 December 2025
Published	: 10 March 2026
Available Onlie	: 12 March 2026

Keywords:
Food safety;
multispecies probiotics;
probiotic efficacy;
shrimp aquaculture;
V. parahaemolyticus

ABSTRACT

Global seafood production relies on shrimp aquaculture, but disease outbreaks and diminishing water quality limit productivity in intensive farming methods. Probiotics are advertised as eco-friendly methods to improve shrimp health and pond performance, but field-scale evidence, particularly from multispecies formulations, is scarce. This study aims to evaluate the effectiveness of multispecies probiotics on the health status, production performance, and water quality of *P. vannamei* shrimp farming at a field scale. *P. vannamei* post-larvae were reared in maintenance ponds. The shrimp seeds were stocked in 10 plots (50×50×2 m^2) and acclimated for 7 days. Multispecies probiotics were administered after acclimatization, and health status parameters, production performance, and water quality were measured. The results indicated that multispecies probiotics combined with molasses and fermented bran were ineffective in preventing *V. parahaemolyticus growth*, as evidenced by the health status of *P. vannamei*. However, multispecies probiotics improved production performance and water quality through the end of the maintenance period. This study highlights the effectiveness of multispecies probiotics in enhancing the production performance and water quality. Further research is needed to understand the mechanism of action of these probiotics in Vannamei shrimp on a field scale.

How to Cite this Article Siregar, L.E.S., S. Rahardjo, Tb.H. Rahayu & P.A. Wiradana. 2026. Field testing of multispecies probiotics on health status, water quality, and productivity of pacific white shrimp (Penaeus vannamei). Jurnal Perikanan Universitas Gadjah Mada. 28 (1): 09-20. https://doi.org/10.22146/jfs.111975

INTRODUCTION

Aquaculture is the most productive industry in the fisheries sector, and the high demand for aquaculture products necessitates the implementation of intensified farming systems while seeking more environmentally friendly alternatives (Egger & Ahern, 2022). Antibiotics were widely used in the 20th century to treat diseases and prevent economic losses (Salam *et al.,* 2023). However, the downside of antibiotics is their ability to exert selective pressure, leading to bacterial resistance and horizontal gene transfer among bacterial communities in reservoirs, which further spreads these genes into the aquatic environment (Serwecińska, 2020). Antibiotic residues in seafood and other derivative products negatively impact human health (Okocha *et al.,* 2018).

Pacific white shrimp (*Penaeus vannamei*) is the most productive aquaculture commodity in Asia, including Indonesia (Rahardjo *et al.,* 2022; Sani *et al.,* 2022; Wiradana *et al.,* 2019). The Intensification and expansion of farming activities have reduced the quality and productivity of *P. vannamei* production by decreasing disease immunity (El-Saadony *et al.,* 2022). Disease outbreaks are often associated with uneven seed stock and feed development, as well as the unwise use of chemicals and antibiotics. Infectious diseases caused by viruses, bacteria, or parasites are detrimental to commercial shrimp farming activities and result in significant economic losses (Zhang *et al.,* 2016).

Functional feed additives have been recommended in modern *P. vannamei* farming activities as a viable biotechnology technique to address infectious diseases and antibiotic resistance (Srirengaraj *et al.,* 2023). Probiotics are proposed as a practical solution because they are safer and more environmentally friendly (Hossain *et al.,* 2017). Shifting towards the use of probiotics not only addresses existing issues but also aligns with sustainable and responsible aquaculture operations (Verdegem *et al.,* 2023). Several research reports have revealed the effectiveness of probiotics in reducing pathogen hazards through competition for nutrients and adhesion sites in the host's gut, synthesis of antimicrobial compounds, and modulation of the host's immune system (Puvanendran *et al.,* 2021). One study reported that six isolates of lactic acid bacteria (LAB) showed potential as probiotic candidates, providing protection and enhancing the growth performance of *P. vannamei* infected with *Vibrio harveyi* (Cai *et al.,* 2022). The probiotic *Bacillus subtilis* AQAHBS001 has been reported to improve water quality in *P. vannamei* rearing (Kewcharoen & Srisapoome, 2019). A yeast-based probiotic agent, *Rhodotorula paludigena* CM33, developed for *P. vannamei*, was able to enhance essential nutritional values, including crude protein and crude fat content (Sriphuttha *et al.,* 2023). Fermented lemon peel waste *(Citrus lemon)* with probiotic *L. plantarum* has potential as a feed additive for *P. vannamei* by increasing body weight, reducing feed conversion ratios, enhancing survival rates, and providing resistance to *V. alginolyticus* infections *(*Lee *et al.,* 2024).

The use of monospecies probiotics may only improve the health of *P. vannamei* but does not affect the quality of rearing water (Amenyogbe, 2023). For example, probiotics such as *Bacillus* spp. and *Lactobacillus* spp. are known to colonize the shrimp gut and produce various substances like enzymes, antimicrobial peptides, and long-lasting spore layers (Chien *et al.,* 2020; Kewcharoen & Srisapoome, 2019). Nevertheless, monospecies probiotics generally exert their effects solely within the host organism, without affecting the rearing water quality for P. vannamei. Furthermore, the utilization of probiotics in numerous studies remains confined to laboratory settings.

The application of multispecies probiotics (*L. fermentum*, *L. plantarum*, *B. subtilis*, *Pseudomonas putida*, as well as *Nitrosomonas* sp. and *Nitrobacter* sp.) on the production performance, water quality, and health status of *P. vannamei* has not yet been reported. Field-scale application of multispecies probiotics is also limited and must consider several important factors, such as bacterial viability, preparation effectiveness, and safety for host health (Ramadhani *et al.,* 2019). This study aims to monitor the effectiveness of multispecies probiotics on the health status, water quality, and productivity of *P. vannamei* at a field scale. This study can contribute to a better understanding of how to apply multispecies probiotics in *P. vannamei* aquaculture at the field scale.

MATERIALS AND METHODS

Study area and preparations

The study was conducted from February to May 2023 at the *P. vannamei* farm owned by PT. Semeru Teknik, Tukak Sadai District, Bangka Belitung Province, Indonesia. This *P. vannamei* farm implements an intensive farming system with square rearing containers measuring 50 m in length, 50 m in width, and 2 m in height. The rearing ponds were lined with HDPE plastic and were equipped with two aerator paddles for dissolved oxygen supply, ten 8-inch water pumps, and 6-meter siphon hoses used to remove feed residue deposits from the pond bottom. *P. vannamei* post larvae (PL) 7 were stocked in the rearing ponds and fed commercial feed containing a minimum of 46% protein from PT. Charoen Pokphand during the acclimatization period (7 days).

Research design

This study employed a single-treatment design with 10 replications (in 10 *P. vannamei* rearing pond plots) that received multispecies probiotics. The effectiveness of the addition of multispecies probiotics on the health of *P. vannamei* shrimp was observed through the dynamics of *V. parahaemolyticus* in the hepatopancreas and intestine, the percentage of Aggregated Transformed Microvilli (ATM), and production performance (average body weight, average daily growth, survival rate, and feed conversion ratio). The effectiveness of multispecies probiotics was also assessed based on water quality parameters, including temperature, dissolved oxygen, pH value, salinity, alkalinity, nitrite, phosphate, total organic matter, total vibrio count, and total bacterial count (Marwiyah *et al.,* 2019; Sani *et al.,* 2020; Wiradana *et al.,* 2023).

Preparation of rearing ponds and stocking of P. vannamei

The test containers were square earthen ponds lined with high-density polyethylene (HDPE) and measured 50 m in length, 50 m in width, and 1.5 m in height. The total tank volume was 3,750 m^3, with 10 tanks used. The rearing water height was 1.3 m, and the rearing water volume was 3,250 m^3. The rearing ponds were equipped with 8 paddles, one on each side. Seawater was distributed via a pump and 10-inch pipes to the rearing tank and was sterilized with UV light before being used in the main rearing pond. The water was also chemically sterilized with copper sulfate at 1.2 ppm for 24 hours and disinfected at 4 ppm for 48 hours. Specific pathogen-free (SPF) *P. vannamei* shrimp at PL-7 size were stocked at 200 shrimp/m^2 using an intensive system. Acclimatization was carried out for 7 days, and commercial feed was provided *ad libitum*. Stock *P. vannamei* seeds in the late afternoon (4:00-6:00 PM) to reduce thermal stress and align with peak dissolved oxygen levels (Wiradana *et al*., 2023).

Feed preparation and application

Fermented feed and rearing-water regimens were formulated utilizing multispecies probiotics. For feed fermentation, 2 L of molasses was combined with 500 mL of probiotics, diluted with water to a total volume of 25 L, and incubated for 24 hours. The fermented solution was used as a feed additive at 200 mL per kilogram of feed. The multispecies probiotics included in the feed comprised *Lactobacillus fermentum, Lactobacillus plantarum, Bacillus subtilis*, and *Pseudomonas putida*. The probiotic-enriched feed was provided five times daily at a feeding rate corresponding to 20% of the average body weight of *Penaeus vannamei*, following previously established protocols (Yudiati *et al*., 2016; Wiradana *et al*., 2019; Yudiati *et al*., 2019).

In addition to their use in dietary applications, probiotics were employed in rearing media to maintain water quality stability through microbial degradation. Briefly, 25 kg of bran was combined with 1,000 mL of probiotics and 35 liters of water, then incubated for 24 hours prior to being uniformly distributed into the rearing pools at 09:00 a.m. The aquatic probiotics primarily comprised *Nitrosomonas* sp. and *Nitrobacterium* sp. and were administered continuously for 14 days at a dosage of 500 mL of probiotics mixed with 5 L of molasses.

Observation of P. vannamei health status

Health status observation of *P. vannamei* is performed by sampling five prawns from each rearing pond plot. The *P. vannamei* specimens are collected live and transferred to sample containers for laboratory examination. Observations were performed on the intestine, lipids, and tubular structures of the *P. vannamei* hepatopancreas. Briefly, the hepatopancreas of each sample was excised by exposing the carapace. The hepatopancreas is subsequently aseptically excised with sterile forceps and positioned on a glass slide. The intestine is excised by severing a segment at the base of the tail and carefully removed with sterile forceps. The intestine is washed with sterile distilled water and subsequently positioned on a glass slide. The intestinal health, lipid deposition, and tubular architecture of the hepatopancreas were evaluated separately for each crustacean (Sani *et al*., 2020). The percentage change for each parameter was calculated relative to the control group using the following equation:

Percentage Change (%) = [Treatment Mean Score - Control Mean Score) / Control Mean Score] × 100

Detection of V. parahaemolyticus

The presence of *V. parahaemolyticus* was identified by culturing the *P. vannamei* hepatopancreas sample on sterile ChromAgar media. Briefly, 18.6 g of ChromAgar media was weighed and placed into a 300 mL Erlenmeyer flask, and 150 mL of physiological solution is added. The media solution was sterilized in an autoclave at 121 °C for 15 minutes. P. vannamei hepatopancreas samples were randomly collected using surgical scissors and tweezers. The hepatopancreas was removed by opening the carapace of the *P. vannamei* between the cephalothorax and abdomen. The hepatopancreas samples were then placed in microtubes and labeled. The hepatopancreas samples in the microtubes were diluted with physiological saline and homogenized with a vortex. Subsequently, 100 µL of hepatopancreas homogenate was taken using a micropipette and inoculated on ChromAgar media, homogenized, and incubated at 30°C for 24 hours (Ha *et al*., 2023).

Counting of Vibrio parahaemolyticus colonies

The total number of *Vibrio parahaemolyticus* in the hepatopancreas was counted after a 2-hour incubation period. *V. parahaemolyticus* colonies that grow on the media were marked by purple-colored colonies. The total bacteria count of *V. parahaemolyticus* was calculated using the following equation:

$$Total\ V. parahaemolyticus\left(\frac{CFU}{ml}\right) = \frac{number\ of\ Vibrio\ colonies \times 10^4 \times weight\ of\ hepatopancreas}{number\ of\ shrimp\ samples}$$

Observation of production performance

Production performance data were obtained using the following equations:

Average body weight (ABW)

The average body weight per shrimp was calculated using the equation from Wiradana *et al*. (2019):

$$ABW\left(\frac{g}{shrimp}\right) = \frac{weight\ of\ shrimp\ weighed\ (g)}{number\ of\ shrimp\ weighed\ (g)}$$

Average daily growth (ADG)

The daily weight gain of shrimp over a period was calculated using the equation from Nederlof et *al*. (2023):

$$ADG\left(\frac{g}{day}\right) = \frac{ABW2\left(\frac{g}{shrmp}\right) - ABW1\left(\frac{g}{shrimp}\right)}{\Delta t(days)}$$

Survival rate (SR)

The survival rate was calculated to determine the final shrimp population using the equation from Wiradana *et al*. (2019):

$$SR(\%) = \frac{final\ shrimp\ population}{initial\ shrimp\ population} \times 100\%$$

Feed conversion ratio (FCR)

The FCR was calculated to determine the amount of feed consumed by shrimp converted into body weight using the equation from Lee *et al.* (2023):

$$FCR = \frac{Total\ feed\ consumed\ (kg)}{biomass\ (kg)}$$

Water quality measurement

The water quality of the vannamei shrimp rearing ponds was measured by assessing physical, chemical, and biological parameters, including dissolved oxygen (DO), pH value, salinity, temperature, alkalinity, nitrite, total ammonia, total organic matter, total Vibrio count (TVC), and total bacterial count (TBC) (Riandi *et al.,* 2021; Wiradana *et al.,* 2022).

Data analysis

Data analysis was conducted using descriptive statistics. In this study, the research design was uncontrolled, and only one treatment was used to assess the effectiveness of probiotics and fermentation materials on the presence of Vibrio parahaemolyticus and on the health and growth of vannamei shrimp at a field scale. The data were analyzed using descriptive statistics, including the mean and standard deviation. Water quality measurement data were compared with the National Standard of Indonesia (SNI) No. 8008:2014 regarding vannamei shrimp production. The data were then presented in tables, graphs, and charts to help visualize the data and results.

RESULTS AND DISCUSSION

The use of probiotics to control pathogenic bacteria in aquaculture has been widely studied in recent years, showing promising results in some cases (Simón *et al.,* 2021). However, the effectiveness of probiotics can vary with several factors, including the specific bacterial strains used, dosages and application methods, and environmental conditions in shrimp rearing ponds, particularly at the field scale. The fermentation method used in this study involved molasses and bran. Fermentation can produce various biological products beneficial for aquaculture, including probiotics, enzymes, and bioactive compounds (Manan *et al.,* 2023). However, the efficacy of fermentation in reducing pathogenic bacteria in aquaculture has not been extensively studied. The findings from this study indicate that multispecies probiotic and fermentation treatments were ineffective at reducing Vibrio parahaemolyticus levels in shrimp ponds. This is consistent with previous research reports, including a study that found that probiotic treatments containing *Lactobacillus plantarum* and *Bacillus amyloliquefaciens* were ineffective at reducing AHPND infection in *Penaeus vannamei* (Amiin *et al.,* 2023).

The presence of *V. parahaemolyticus* in the hepatopancreas of vannamei shrimp was identified in several rearing ponds. The probiotic treatment used in this study contained *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Bacillus subtilis*, and *Pseudomonas putida*. However, it did not reduce the presence of *V. parahaemolyticus* in the hepatopancreas of vannamei shrimp. Consequently, mortality of vannamei shrimp was still observed in some rearing ponds due to Acute Hepatopancreatic Necrosis Disease (AHPND) caused by *Vibrio parahaemolyticus* infection. The identification results of the presence of *V. parahaemolyticus* in the hepatopancreas of vannamei shrimp in each rearing pond are shown in Table 1.

Table 1 shows that on the 35th day of the rearing period, some vannamei shrimp rearing plots were positive for *V. parahaemolyticus* infection. Plot C10 was identified as being infected with *V. parahaemolyticus* at 1.717 CFU/g, higher compared to ponds C1 and C7. Meanwhile, the

Table 1. Identification results of *V. parahaemolyticus* in the hepatopancreas of *P. vannamei*.

Ponds	DOC	Vibrio parahaemolyticus (CFU/g) *
C1	36	824*
C2	36	0
C3	36	0
C4	36	0
C5	36	0
C6	35	0
C7	35	237*
C8	35	0
C9	35	0
C10	35	1717*

Note: CFU = Colony Forming Unit; DOC = Days of Culture.

other rearing ponds yielded negative results upon examination using CHROMAgar media. These results indicate that the use of probiotics and fermentation materials has not been effective in preventing V. parahaemolyticus infection naturally at the field scale. The presence of *Vibrio parahaemolyticus* in the hepatopancreas of vannamei shrimp is correlated with a high mortality rate observed within a short period in rearing plots. Mortality in some ponds was observed on the 40th day of rearing, consistent with the onset of AHPND symptoms (Kumar *et al.,* 2021)as a high-protein animal food commodity, are one of the fastest growing food producing sectors in the world. It has emerged as a highly traded seafood product, currently exceeding 8 MT of high value. However, disease outbreaks, which are considered as the primary cause of production loss in shrimp farming, have moved to the forefront in recent years and brought socio-economic and environmental unsustainability to the shrimp aquaculture industry. Acute hepatopancreatic necrosis disease (AHPND. Despite the addition of multispecies probiotics and fermentation using molasses and bran, *Vibrio parahaemolyticus* was still identified in some ponds, indicating that eliminating pathogens completely from the shrimp pond environment remains challenging. Additionally, fermentation can create a conducive environment for the growth of harmful bacteria, especially if not properly controlled (Zokaeifar *et al.,* 2012).

The ineffectiveness of the combination of probiotics and the fermentation process in preventing the growth of *Vibrio parahaemolyticus* can be attributed to several factors, such as improper dosage or application timing, suboptimal environmental conditions, or the presence of other bacterial strains that can interfere with the performance of multispecies probiotics (Grumet *et al.,* 2020). Controlling *Vibrio parahaemolyticus* in shrimp ponds requires a synergistic approach that considers not only probiotics and fermentation but also strategies such as water quality management, biosecurity measures, and selective breeding of disease-resistant shrimp (Tran *et al.,* 2013; Wiradana *et al.,* 2023).

The probiotics used in the study contained *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Bacillus subtilis*, and *Pseudomonas putida*, combined with molasses and bran fermentation. *Lactobacillus fermentum* and *Lactobacillus plantarum*, which are lactic acid bacteria, have shown promising results in improving growth performance and disease resistance in aquatic organisms (Dawood *et al.,* 2015). *Bacillus subtilis*, a spore-forming bacterium, has been reported to produce antimicrobial substances and stimulate the host's immune system (Kamaruddin *et al.,* 2021). *Pseudomonas putida*, known for its ability to degrade organic matter, has potential inhibitory effects on pathogen growth (Jamal *et al.,* 2019). The fermentation process using molasses and bran aims to provide a suitable environment for the growth and metabolic activity of beneficial microorganisms (Khanjani *et al.,* 2023). Fermentation can increase the production of antimicrobial compounds, organic acids, and other bioactive substances that contribute to disease control and gut health improvement (Zhang *et al.,* 2021). Despite the inclusion of probiotic strains and fermentation, the presence of Vibrio parahaemolyticus and the occurrence of mild-to-moderate intestinal infections indicate that the methods employed were ineffective in reducing pathogens or restoring shrimp gut health.

The hepatopancreas functions as a crucial organ for lipid metabolism, including lipid synthesis, storage, and mobilization in shrimp (Wang *et al.,* 2014). Infection by *Vibrio parahaemolyticus* causes alterations in lipid metabolism in vannamei shrimp, including decreased lipid accumulation in the hepatopancreas, triggering an inflammatory response and disrupting lipid metabolism-related enzymes (Tassanakajon *et al.,* 2013). The observed reduction in lipid content indicates the impact of AHPND on lipid metabolism in vannamei shrimp (Xu *et al.,* 2018). Disruption of lipid metabolism can result in reduced energy reserves and affect growth rates (Wiradana *et al.,* 2022). A study on shrimp found that *V. parahaemolyticus,* causing AHPND, disrupts lipid metabolism and acid synthesis (Ng *et al.,* 2018). *V. parahaemolyticus* infection can cause changes in lipids because many pathogens can modulate shrimp lipid metabolism (Wang *et al.,* 2022).

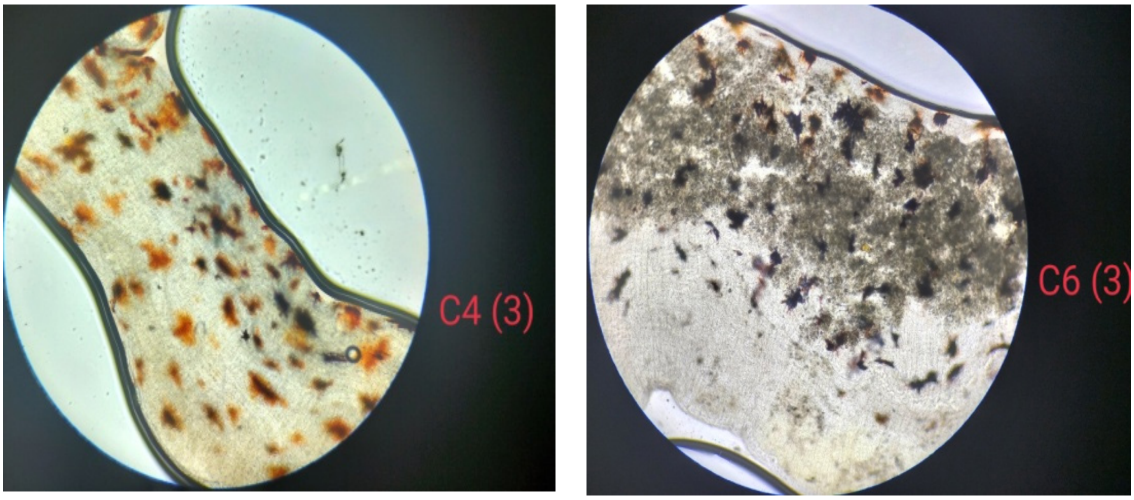
The health condition of vannamei shrimp was observed based on lipid, tubule structure, and morphometric condition of the intestine. The health observation of shrimp was conducted on the 35th day of the rearing period. The health observation results of vannamei shrimp are shown in Table 2.

Macroscopic observations of vannamei shrimp intestines showed mild to moderate infections. This indicates that the administration of multispecies probiotics has not been effective in maintaining the overall intestinal health of vannamei shrimp at a field scale. Infected intestines were marked by the appearance of red spots on the intestines (Figure 1). Similarly, the lipid condition in the hepatopancreas of vannamei shrimp ranged from an average of 70% to 100%. The decrease in lipid percentage is suspected to be due to *V. parahaemolyticus* infection causing AHPND, marked by the reduction of lipid points in the tubules, as seen in Figure 2B.

Damage to the tubule shape and structure indicates that V. parahaemolyticus infection induces structural changes in the tubules. Observations showed that tubule shrink-

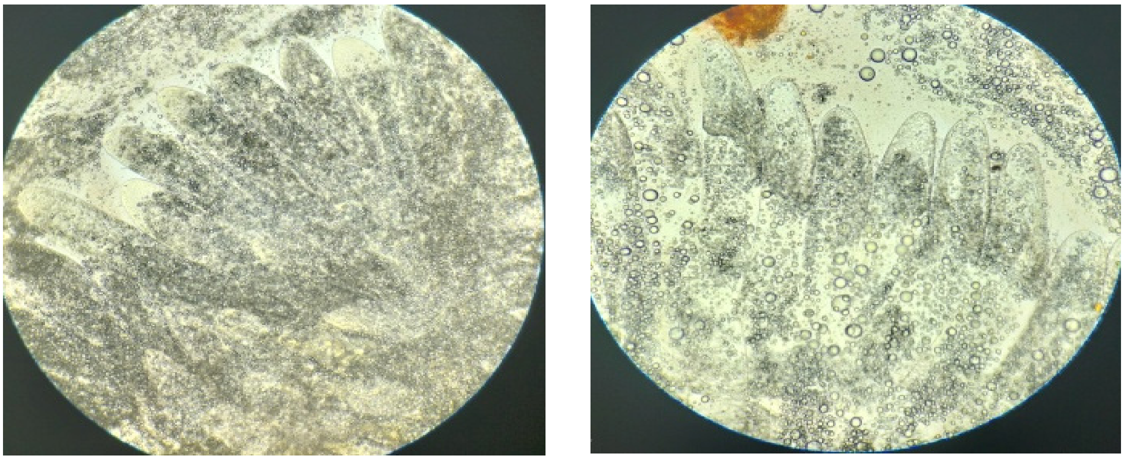
Table 2. Observation results of *P. vannamei* health.

No.	Sample	Intestinal Condition	Lipid (%)	Tubule Condition
1.	C1	Full, normal intestine	80-100	Good
2.	C2	Full, mild-moderate infection intestine	80-100	Good Shrinkage 5%
3.	C3	Full, normal-mild infection intestine	80-90	Good Shrinkage 5%
4.	C4	Partially full-empty, normal-moderate infection intestine	90-100	Good
5.	C5	Full, normal intestine	70-80	Good Shrinkage 5%
6.	C6	Full, mild infection intestine	80	Shrinkage 5-10%
7.	C7	Partially full-empty, normal intestine	100	Good Shrinkage 10%
8.	C8	Partially full-empty, normal intestine	80-100	Shrinkage 5-10%
9.	C9	Partially full-empty, normal-moderate infection intestine	80-90	Shrinkage 5-10%
10.	C10	Full-empty, normal-mild infection intestine	75-100	Shrinkage 10-20%



A B

Figure 1. Fresh preparate of *P. vannamei intestine*. A. intestine indicated to be infected by *V. parahaemolyticus,* and B. normal intestine.

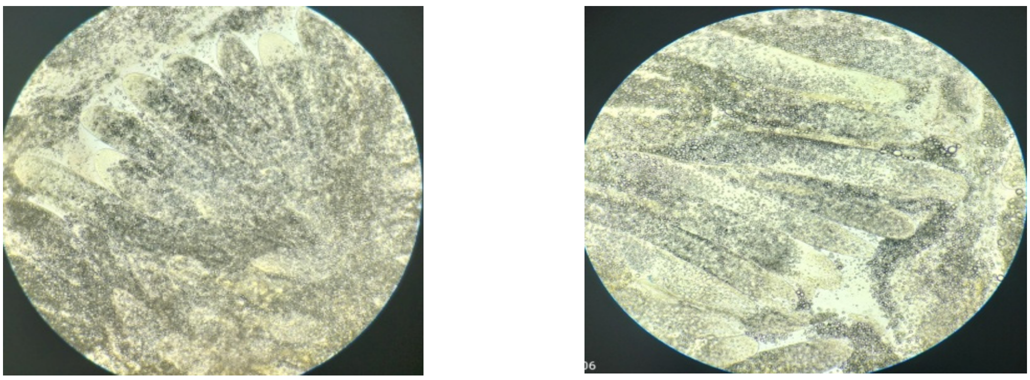


A B

Figure 2. Lipid droplet in the hepatopancreas of *P. vannamei*. A. normal lipid points and B. lipid points reduced by up to

age occurred under normal conditions, with a reduction of up to 20%. The ineffective use of multispecies probiotic methods and suboptimal aquaculture environments in preventing natural *V. parahaemolyticus* infections led to tubule shrinkage in the hepatopancreas organ (Figure 3). Aggregated Transformed Microvilli (ATM) were also observed in the hepatopancreas, ranging from 5% to 10%. This observation indicates structural changes in the microvilli of *Penaeus vannamei* in response to treatment interventions and rearing environment conditions (Figure 4). The percentage of ATM observations in the samples is shown in Table 3.

Table 3 shows the percentage of ATM ranging from 5-10% in the observed samples. The presence of ATM in the hepatopancreas indicates cell damage in the hepatopa-



A B

Figure 3. Tubule condition of *P. vannamei*. A. normal tubule structure and B. shrunken tubules.

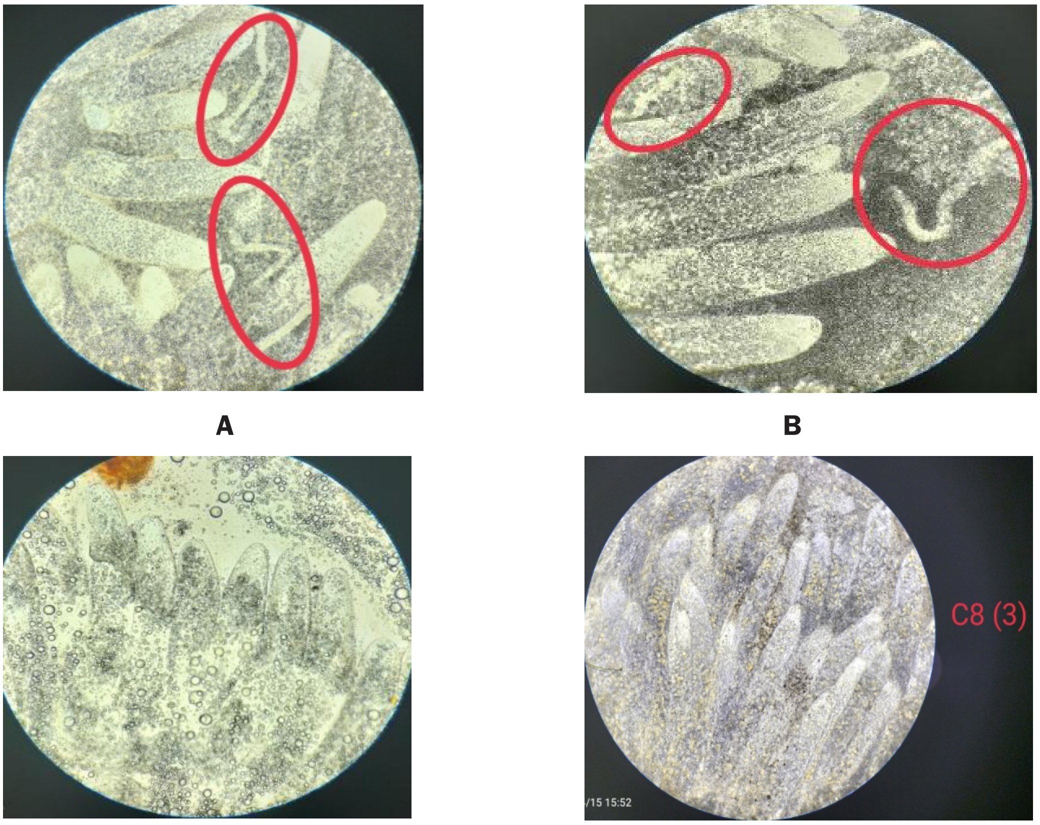


Figure 4. Condition of Aggregated Transform Microvilli (ATM) in the hepatopancreas of *P. vannamei*. A and B. ATM present in the hepatopancreas; C and D. no ATM present in the hepatopancreas.

ncreatic tubules, leading to transformation in the tubule structure. The formation of ATM is believed to be triggered by bacterial toxin secretion, which alters gene expression involved in microvilli formation. Aggregated Transformed Microvilli (ATM) is a term used to describe the abnormal clustering and aggregation of microvilli in the hepatopancreas of vannamei shrimp infected with Acute Hepatopancreatic Necrosis Disease (AHPND), also known as Early Mortality Syndrome (EMS).

Tubule shrinkage occurs due to epithelial peeling and necrosis in the tubules of the hepatopancreas in the late stages of *V. parahaemolyticus* infection (*Soto-Rodriguez et al.,* 2015). Acute Hepatopancreatic Necrosis Disease (AHPND) causes tubule deformation in the hepatopancreas of shrimp, including vannamei shrimp (Morales-Covarrubias *et al.,* 2018). The PirB toxin, involved in the peeling and damage of epithelial cells in the tubules, is detected in the hepatopancreas and shows histological damage (Zheng *et al.,* 2021). The PirB toxin also targets and damages cells in the hepatopancreas, causing necrosis and structural changes (Soto-Rodriguez *et al.,* 2022). The PirAB toxin disrupts the normal function of hepatopancreas cells, particularly those involved in digestion, absorption, and nutrient metabolism. This disruption interferes with the normal processes of lipid and carbohydrate metabolism in the hepatopancreas (Kumar *et al.,* 2019).

The effectiveness of multispecies probiotics and fermentation materials was also observed based on growth aspects. The growth of vannamei shrimp was analyzed based on sampling data. Sampling was conducted at

Table 3. Percentage of aggregated transform microvilli (ATM).

Sample	Percentage ATM (%)
C1	5
C2	5-10
C3	5-10
C4	5
C5	5
C6	5-10
C7	5
C8	5
C9	5-10
C10	5-10

5-day intervals. The results can be seen in Table 4. The average weight of shrimp during the study showed significant differences in each pond. An increase in average weight in each pond was observed at each sampling period. The final ABW obtained during the study ranged from 3.29-7.96 g/shrimp. Some ponds experienced early harvest due to high mortality caused by *V. parahaemolyticus* infection. The application of probiotics and fermentation materials positively impacted the shrimp's digestive system but was not effective in preventing *V. parahaemolyticus* infection, ultimately affecting shrimp metabolism and growth rates.

The daily weight gain of shrimp during the study exhibited varying growth rates across sampling periods. In the second sampling period, the growth rate ranged from 0.12 to 0.3 g/day. According to SNI-01-7246-2006, the standard value for daily weight gain of shrimp is 0.2 g/day. During the second sampling period, the average weight gain of shrimp remained within a reasonable range relative to the standard value. In the third sampling period, the daily growth rate in pond C3 declined to 0.05 g/day, representing only 0.25 g over five days. The third sampling period, conducted on the 45th day of rearing, showed a decrease in the average daily weight of shrimp, likely due to the spread of *V. parahaemolyticus* infection in the rearing area.

Table 4. Production performance of *P. vannamei* with multi-species probiotics at field scale.

No.	Parameter	Range Value	SNI Standard	Notes
1.	Average Body Weight (ABW)	3.29-7.96 g/shrimp	25 g/shrimp	Not optimal
2.	Average Daily Growth (ADG)	0.04 - 0.39 g/day	0.2 g/day	Not optimal
3.	Survival Rate (SR)	23 - 72%	75%	Not optimal
4.	Feed Conversion Ratio (FCR)	1.02 - 3.23	1.5	Not optimal

Based on the results of this study, the average weight of shrimp increased. According to Suryana *et al.* (2023) Shrimp infected with AHPND generally weigh between 0.2 and 2.5 g. The observed increase in average weight can be attributed to several factors associated with the use of probiotics and fermentation. Probiotics such as *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Bacillus subtilis*, and *Pseudomonas putida* are reported to improve growth performance and feed utilization in various aquatic species (Shawky *et al.,* 2023). The impact of *Vibrio parahaemolyticus* on ADG can be explained by several factors. First, bacterial pathogens can affect the hepatopancreas, the shrimp's main digestive organ, leading to reduced nutrient absorption and digestive disturbances. This results in suboptimal feed conversion into growth, contributing to lower ADG values (Santos *et al.,* 2020). Rearing ponds C3, C4, C5, and C10 were flushed, with mortality reaching 100%. Pond C1 had a survival rate of 44%, the lowest after the ponds that experienced flushing. Pond C2 had the highest survival rate at 72%. All rearing ponds were harvested early due to mortality observed across all ponds. Early harvesting was undertaken as a precaution against increased mortality. This condition creates an environment conducive to bacterial growth and compromises the shrimp's immune system, making them more susceptible to infection and death (Emerenciano *et al.,* 2022).

The survival rate (SR) at the end of the rearing period ranged from 23-72%. Mortality among vannamei shrimp occurred during the rearing period, indicating that V. parahaemolyticus infection caused high mortality within a relatively short period. The occurrence of *V. parahaemolyticus* infection resulted in rapid death, leading to a low survival rate that did not meet the standard. According to SNI-01-7246-2006, the standard survival rate for vannamei shrimp farming is 75%. In addition to the low survival rate, *V. parahaemolyticus* infection also caused early harvesting and flushing in several rearing ponds.

The feed conversion ratio (FCR) relates to the efficiency of feed utilization relative to the total biomass of the shrimp. According to SNI-01-7246-2006, the standard FCR is 1.5. The FCR values in this study ranged from 1.02 to 3.23. An FCR of 1.02 to 1.1 in the rearing ponds was achieved within a rearing period of 48-49 days. The low FCR values were attributable to the short rearing period resulting from early harvesting and indications of cannibalism in the rearing ponds, which contributed to increased shrimp biomass.

Water quality was measured periodically to assess conditions during the rearing period. The measurement results indicated that some water quality parameters were outside the suitable range for vannamei shrimp habitat. The average water quality measurements during the study are shown in Table 5.

A well-maintained rearing environment supports the smooth metabolism and growth of vannamei shrimp. The optimal temperature range for vannamei shrimp farming is 28.5-31.5°C (Marwiyah *et al.,* 2019). Temperature affects appetite and metabolic processes in shrimp. The availability of DO in the rearing ponds was generally adequate. The difference in dissolved oxygen levels between morning and night is due to higher dissolved oxygen levels from morning to noon because of plankton photosynthesis, while at night, plankton do not perform photosynthesis and become oxygen competitors for shrimp (Yoshikawa *et al.,* 2007; Sani *et al.,* 2022).

A low water pH can cause stress in shrimp, resulting in soft shells and reduced survival rates (Wiyoto *et al.,* 2016). Generally, pond water pH is higher in the afternoon than in the morning. This is due to photosynthesis by natural feed, such as phytoplankton, which absorbs CO_2. The decline in pond water pH is caused by the decomposition of organic matter by microorganisms, which releases CO_2 in the process, reducing oxygen concentration and water pH (Supriatna *et al.,* 2023). High salinity can slow shrimp growth as more energy is absorbed for osmoregulation rather than growth (Liu *et al.,* 2024). Nitrite levels increased between the 41^{st} and 48^{th} days of rearing. High nitrite levels are influenced by elevated total ammonia and high organic matter concentrations during the rearing period. The increase in nitrite levels is due to a darker water color from rising organic matter content and poor

Table 5. Water quality measurement results of *P. vannamei* rearing ponds.

No.	Parameter	Result Range	Standard Value	Notes
1.	Temperature	Morning: 27.0°C - 28.9°C Evening: 29.0°C - 31.1°C	Min. 27°C	Suitable
2.	Dissolved Oxygen (DO)	Morning: 5.20 - 5.63 mg/L Evening: 4.40 - 4.80 mg/L	Min. 4 mg/L	Suitable
3.	pH	Morning: 7.65 - 8.98 Afternoon: 8.22 - 9.19	7.5 - 8.5	Less optimal
4.	Salinity	28 - 31 g/L	10 - 32 g/L	Suitable

performance of nitrifying bacteria (Furtado *et al.*, 2016). The availability of ammonia stimulates the activity of ammonia-oxidizing bacteria, thereby increasing nitrite levels. Nitrite (NO_2) at the maximum limit (0.06 mg/L) is the maximum threshold for vannamei shrimp farming (Widigdo *et al.*, 2021).

The need for phosphate for optimal shrimp growth is influenced by the form of nitrogen compounds (Iber & Kasan, 2021). In this study, TOM values were high from the 23rd and 24th days of rearing, reaching 92.93 mg/L, and continued to increase thereafter. High TOM levels (>60 mg/L) indicate declining water quality and can increase the potential for pathogen growth in the rearing environment, including *Vibrio parahaemolyticus* (Urquhart *et al.*, 2016). The total Vibrio count increased significantly from the 5^{th} to the 6^{th} checking period. In the 5th checking period, there was a decrease in total Vibrio. The reduction in total Vibrio during the 5th period was due to the addition of a probiotic dose of 2 mg/L in the rearing medium. Sudden changes in water chemical properties, such as temperature and salinity, as well as high larval density, can accelerate the growth of *Vibrio* sp (Sampaio *et al.*, 2022). The TBC obtained during the rearing period ranged from 1.8 x 10^3 to 5.7 x 10^4 CFU/mL. Total bacteria are influenced by the concentration of organic matter during the rearing period. The TBC values obtained during the study were within the tolerance range. Bacterial density in water exceeding 10^6 CFU/mL can cause 90% mortality in larval and post-larval shrimp and adult shrimp (Kennedy *et al.*, 2006).

CONCLUSION AND RECOMMENDATION

Conclusion

The use of multispecies probiotics containing *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Bacillus subtilis*, and *Pseudomonas putida*, combined with molasses and bran fermentation, has not been effective in preventing the growth of *Vibrio parahaemolyticus* in shrimp ponds. While the administration of multispecies probiotics and fermentation materials has benefits for shrimp health, it has not been effective in preventing *V. parahaemolyticus* infection, ultimately affecting the overall health status of shrimp. The administration of multispecies probiotics and fermentation materials benefits the growth of vannamei shrimp; however, it has not been effective in preventing V. parahaemolyticus infection, which ultimately affects growth and causes mortality within a relatively short period.

Recommendation

Further research is needed to investigate the effectiveness of different probiotic strains, doses, and fermentation methods in controlling *Vibrio parahaemolyticus* and other pathogenic bacteria in vannamei shrimp. Maintaining optimal water quality conditions is crucial to preventing AHPND in *P. vannamei* aquaculture.

AUTHORS' CONTRIBUTIONS

SR and TbHR: Conceptualization, drafting scripts, acquiring projects, and designing. IESS: collecting data, monitoring, and conducting the research. IESS and SR: sampling, observation, and technical laboratory. SR and PAW: Supervision, editing, and review of manuscripts. All authors discussed the results and contributed to the final manuscript.

ACKNOWLEDGEMENT

We extend our gratitude to the management and staff of PT. Semeru Teknik shrimp farm, Bangka Belitung Province, for the opportunity to conduct this research. The author also thanks the Study Program of Aquaculture Technology, Applied Bachelor Program, Fisheries Business Expert Polytechnic, Jakarta and Directorate General of Aquaculture, Ministry of Marine Affairs and Fisheries (KKP), Republic of Indonesia for supporting and providing facilities for the implementation of this research.

REFERENCES

Amenyogbe, E. 2023. Application of probiotics for sustainable and environment-friendly aquaculture management - A review. Cogent Food & Agriculture. 9 (1). https://doi.org/10.1080/23311932.2023.2226425

Amiin, M.K., A.F. Lahay, R.B. Putriani, M. Reza, S.M.E. Putri, M.A.A. Sumon, M.T. Jamal & M.B. Santanumurti. 2023. The role of probiotics in vannamei shrimp aquaculture performance - A review. Veterinary World. 638–649. https://doi.org/10.14202/vetworld.2023.638-649

Cai, X., J. Wen, H. Long, W. Ren, X. Zhang, A. Huang & Z. Xie. 2022. The probiotic effects, dose, and duration of lactic acid bacteria on disease resistance in *Penaeus vannamei*. Aquaculture Reports. 26: 101299. https://doi.org/10.1016/j.aqrep.2022.101299

Chien, C.-C., T.-Y. Lin, C.-C. Chi & C.-H. Liu. 2020. Probiotic, *Bacillus subtilis* E20 alters the immunity of white shrimp, *Penaeus vannamei* via glutamine metabolism and hexosamine biosynthetic pathway. Fish & Shellfish Immunology. 98: 176-185. https://doi.org/10.1016/j.fsi.2020.01.014

Dawood, M.A.O., S. Koshio, M. Ishikawa & S. Yokoyama. 2015. Interaction effects of dietary supplementation of heat-killed *Lactobacillus plantarum* and β-glucan on growth performance, digestibility and immune response of juvenile red sea bream, *Pagrus major*. Fish & Shellfish Immunology. 45 (1): 33-42. https://doi.org/10.1016/j.fsi.2015.01.033

Egger, A & M. Ahern. 2022. The state of world fisheries and aquaculture 2022. FAO. https://doi.org/10.4060/cc0461en

El-Saadony, M.T., A.A. Swelum, M.M. Abo Ghanima, M. Shukry, A.A. Omar, A.E. Taha, H.M. Salem, A.M. El-Tahan, K.A. El-Tarabily & M.E.Abd. El-Hack. 2022. Shrimp production, the most important diseases that threaten it, and the role of probiotics in confronting these diseases: A review. Research in Veterinary Science. 144: 126-140. https://doi.org/10.1016/j.rvsc.2022.01.009

Emerenciano, M.G.C., A.N. Rombenso, F.d.N. Vieira, M.A. Martins, G.J. Coman, H.H. Truong, T.H. Noble & C.J. Simon. 2022. Intensification of penaeid shrimp culture: An applied review of advances in production systems, nutrition and breeding. Animals. 12 (3): 236. https://doi.org/10.3390/ani12030236

Furtado, P.S., M.A.J. Valenzuela, G. Rodriguez-Fuentes, B.R. Campos, W. Wasielesky & G. Gaxiola. 2016. Chronic effect of nitrite on the rearing of the white shrimp *Penaeus vannamei* in two salinities. Marine and Freshwater Behaviour and Physiology. 49 (3): 201-211. https://doi.org/10.1080/10236244.2016.1163837

Grumet, L., Y. Tromp & V. Stiegelbauer. 2020. The development of high-quality multispecies probiotic formulations: From bench to market. Nutrients. 12 (8): 2453. https://doi.org/10.3390/nu12082453

Ha, P.T.H., Q.V.C. Thi, N.P. Thuy & N.T. Luan. 2023. Multi-antibiotics resistance phenotype of pathogenic *Vibrio parahaemolyticus* isolated from acute hepatopancreatic necrosis disease in *Penaeus vannamei* farmed in the Mekong Delta. Journal of the World Aquaculture Society. 54 (4): 1070-1087. https://doi.org/10.1111/jwas.12945

Hossain, M.I., M. Sadekuzzaman & S.-D. Ha. 2017. Probiotics as potential alternative biocontrol agents in the agriculture and food industries: A review. Food Research International. 100: 63-73. https://doi.org/10.1016/j.foodres.2017.07.077

Iber, B.T & N.A. Kasan. 2021. Recent advances in shrimp aquaculture wastewater management. Heliyon. 7 (11): e08283. https://doi.org/10.1016/j.heliyon.2021.e08283

Jamal, M.T., I.A. Abdulrahman, M.Al. Harbi & S. Chithambaran. 2019. Probiotics as alternative control measures in shrimp aquaculture: A review. Journal of Applied Biology & Biotechnology. 7 (3): 69-77. https://doi.org/10.7324/JABB.2019.70313

Kamaruddin, A., M. Nurhudah, D. Rukmono & A. Wiradana. 2021. Potential of probiotics *Bacillus subtilis* to reduce ammonia levels, *Vibrio* sp. abundance, and increased production performance of seaworm (*Nereis* sp.) under laboratory scale. Iraqi Journal of Veterinary Sciences. 35 (4): 757-763. https://doi.org/10.33899/ijvs.2021.128408.1572

Kennedy, B., M.N. Venugopal, I. Karunasagar & I. Karunasagar. 2006. Bacterial flora associated with the giant freshwater prawn *Macrobrachium rosenbergii*, in the hatchery system. Aquaculture. 261 (4): 1156-1167. https://doi.org/10.1016/j.aquaculture.2006.09.015

Kewcharoen, W & P. Srisapoome. 2019. Probiotic effects of *Bacillus* spp. from pacific white shrimp (*Penaeus vannamei*) on water quality and shrimp growth, immune responses, and resistance to *Vibrio parahaemolyticus* (AHPND strains). Fish & Shellfish Immunology. 94: 175-189. https://doi.org/10.1016/j.fsi.2019.09.013

Khanjani, M.H., L.O.B. da Silva, G.K. Fóes, F.do.N. Vieira, M.A. Poli, M. Santos & M.G.C. Emerenciano. 2023. Synbiotics and aquamimicry as alternative microbial-based approaches in intensive shrimp farming and biofloc: Novel disruptive techniques or complementary management tools? A scientific-based overview. Aquaculture. 567: 739273. https://doi.org/10.1016/j.aquaculture.2023.739273

Kumar, V., L.De. Bels, L. Couck, K. Baruah, P. Bossier & W.V den. Broeck. 2019. PirABVP toxin binds to epithelial cells of the digestive tract and produce pathognomonic AHPND lesions in germ-free brine shrimp. Toxins. 11 (12): 717. https://doi.org/10.3390/toxins11120717

Kumar, V., S. Roy, B.K. Behera, P. Bossier & B.K. Das. 2021. Acute hepatopancreatic necrosis disease (AHPND): Virulence, pathogenesis and mitigation strategies in shrimp aquaculture. Toxins. 13 (8): 524. https://doi.org/10.3390/toxins13080524

Lee, M.-C., C.-T. Lo, T.H. Ho, L.-L. Chen, F.-H. Nan, H.-C. Lai, E. Wangkahart & P.-T. Lee. 2023. Assessment of *Bacillus subtilis* fermented *Caulerpa microphysa* byproduct as feed additive on the growth performance, immune regulation and disease resistance of white shrimp (*Penaeus vannamei*). Fish & Shellfish Immunology. 142: 109134. https://doi.org/10.1016/j.fsi.2023.109134

Lee, Y.-C., C.-C. Chang, Y.-H. Lin & Y.-H. Lin. 2024. Effect of fermented lemon peel as a functional feed additive on growth, non-specific immune responses, and *Vibrio alginolyticus* resistance in whiteleg shrimp, *Penaeus vannamei*. Aquaculture Reports. 34: 101918. https://doi.org/10.1016/j.aqrep.2024.101918

Liu, F., J. Sun, J. Long, L. Sun, C. Liu, X. Wang, L. Zhang, P. Hao, Z. Wang, Y. Cui, R. Wang & Y. Li. 2024. Assessing the interactive effects of high salinity and stocking density on the growth and stress physiology of the pacific white shrimp *Penaeus vannamei*. Fishes. 9 (2): 62. https://doi.org/10.3390/fishes9020062

Manan, H., M. Jalilah, F. Fauzan, M. Ikhwanuddin, A. Amin-Safwan, N.S. Abdullah, M. Nur-Syahirah & N.A. Kasan. 2023. Recent developments in aquaculture - A review. Annals of Animal Science. 23 (3): 663-680. https://doi.org/10.2478/aoas-2023-0044

Marwiyah, U.C., G. Mahasri, R.E. Ratnasari & P.A. Wiradana. 2019. Total plate count and identification of Vibrio in pacific white shrimp (*Litophenaeus vannamei*) from ponds and in those exposed to immunogenic protein membrane *Zoothamnium penaei*. IOP Conference Series: Earth and Environmental Science. 236 (1). https://doi.org/10.1088/1755-1315/236/1/012087

Morales-Covarrubias, M.S., J. Cuellar-Anjel, A. Varela-Mejias & C. Elizondo-Ares. 2018. Shrimp bacterial infections in Latin America: A review. Asian Fisheries Science. 31S. https://doi.org/10.33997/j.afs.2018.31.S1.005

Nederlof, M.A.J., S.J. Kaushik & J.W. Schrama. 2023. Effect of different types of bacterial single cell protein on feed intake, digestibility, growth and body composition of Pacific white shrimp (*Penaeus vannamei*). Aquaculture Reports. 33: 101830. https://doi.org/10.1016/j.aqrep.2023.101830

Ng, T.H., C. Lu, S. Lin, C. Chang, L.H. Tran, W. Chang, C. Lo & H. Wang. 2018. The Rho signalling pathway mediates the pathogenicity of AHPND-causing V. parahaemolyticus in shrimp. Cellular Microbiology. 20 (8). https://doi.org/10.1111/cmi.12849

Okocha, R.C., I.O. Olatoye & O.B. Adedeji. 2018. Food safety impacts of antimicrobial use and their residues in aquaculture. Public Health Reviews. 39 (1): 21. https://doi.org/10.1186/s40985-018-0099-2

Puvanendran, V., I. Rud, M.S.W. Breiland, J.A. Arnesen & L. Axelsson. 2021. Probiotic Carnobacterium divergens increase growth parameters and disease resistance in farmed Atlantic cod (*Gadus morhua*) larvae without influencing the microbiota. Aquaculture. 532: 736072. https://doi.org/10.1016/j.aquaculture.2020.736072

Rahardjo, S., M. Vauza, D. Rukmono & P.A. Wiradana. 2022. Supplementation of hairy eggplant (*Solanum ferox*) and bitter ginger (*Zingiber zerumbet*) extracts as phytobiotic agents on whiteleg shrimp (*Penaeus vannamei*). Journal of Advanced Veterinary and Animal Research. 9 (1): 78. https://doi.org/10.5455/javar.2022.i571

Ramadhani, D.E., W. Widanarni & S. Sukenda. 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

Riandi, M.I., R.J.K. Susilo, M.D. Sani, A.Y. Maharani, A. Soegianto, T.W.C. Putranto & P.A. Wiradana. 2021. Surveillance of Vibrio and blue-green algae in intensive system of pacific white shrimp (*Penaeus vannamei*) in Situbondo Regency, East Java, Indonesia. Poll Res. 40 (2): 611-616.

Salam, M.A., M.Y. Al-Amin, M.T. Salam, J.S. Pawar, N. Akhter, A.A. Rabaan & M.A.A. Alqumber. 2023. Antimicrobial resistance: A growing serious threat for global public health. Healthcare. 11 (13): 1946. https://doi.org/10.3390/healthcare11131946

Sampaio, A., V. Silva, P. Poeta & F. Aonofriesei. 2022. *Vibrio* spp.: Life strategies, ecology, and risks in a changing environment. Diversity. 14 (2): 97. https://doi.org/10.3390/d14020097

Sani, M.D, A.Y. Maharani, M.I. Riandy, R.J.K. Susilo, P.A. Wiradana & A. Soegianto. 2020. Monitoring of population density of Vibrio sp. and health condition of hepatopancreas pacific white shrimp (*Penaeus* vannamei) cultivated with intensive systems in Bulukumba Regency, South Sulawesi, Indonesia. Ecology, Environment and ConservationEcology, Environment and Conservation. 26 (3): 1271-1275.

Sani, M.D., A.Y. Maharani, M.I. Riandy, R.J.K. Susilo, P.A. Wiradana & A. Soegianto. 2020. Monitoring population density of *Vibrio* sp. and health condition of hepatopancreas pacific white shrimp (*Penaeus vannamei*) cultivated with intensive systems in Bulukumba Regency, South Sulawesi, Indonesia. Ecology, Environment and Conservation. 26 (3): 1271-1275.

Sani, M.D., P.A. Wiradana, A.Y. Maharani, R.E. Mawli & A.T. Mukti. 2022. The dominance and proportions of plankton in pacific white shrimp (*Penaeus vannamei*) ponds cultivated with the intensive system in Bulukumba Regency, South Sulawesi, Indonesia. IOP Conference Series: Earth and Environmental Science. 1036 (1): 012057. https://doi.org/10.1088/1755-1315/1036/1/012057

Santos, H.M., C.-Y. Tsai, K.R.A. Maquiling, L.L. Tayo, A.R. Mariatulqabtiah, C.-W. Lee & K.P. Chuang. 2020. Diagnosis and potential treatments for acute hepatopancreatic necrosis disease (AHPND): A review. Aquaculture International. 28 (1): 169-185. https://doi.org/10.1007/s10499-019-00451-w

Serwecińska, L. 2020. Antimicrobials and antibiotic-resistant bacteria: A risk to the environment and to public health. Water. 12 (12): 3313. https://doi.org/10.3390/w12123313

Shawky, A., I.M. Abd El-Razek, R.S. El-Halawany, A.I. Zaineldin, A.A. Amer, M.S. Gewaily & M.A.O. Dawood. 2023. Dietary effect of heat-inactivated *Bacillus subtilis* on the growth performance, blood biochemistry, immunity, and antioxidative response of striped catfish (*Pangasianodon hypophthalmus*). Aquaculture. 575: 739751. https://doi.org/10.1016/j.aquaculture.2023.739751

Simón, R., F. Docando, N. Nuñez-Ortiz, C. Tafalla & P. Díaz-Rosales. 2021. Mechanisms used by probiotics to confer pathogen resistance to teleost fish. Frontiers in Immunology. 12. https://doi.org/10.3389/fimmu.2021.653025

Soto-Rodriguez, S.A., B. Gomez-Gil, R. Lozano-Olvera, M. Betancourt-Lozano & M.S. Morales-Covarrubias. 2015. Field and experimental evidence of *Vibrio parahaemolyticus* as the causative agent of acute hepatopancreatic necrosis disease of cultured shrimp (*Penaeus vannamei*) in Northwestern Mexico. Applied and Environmental Microbiology. 81 (5): 1689-1699. https://doi.org/10.1128/AEM.03610-14

Soto-Rodriguez, S.A., R. Lozano-Olvera, M.G. Ramos-Clamont, E. Zenteno, J.L. Sánchez-Salgado, N. Vibanco-Pérez & K.G. Aguilar-Rendón. 2022. New insights into the mechanism of action of PirAB from *Vibrio Parahaemolyticus*. Toxins. 14 (4): 243. https://doi.org/10.3390/toxins14040243

Sriphuttha, C., S. Limkul, J. Pongsetkul, T. Phiwthong, A. Massu, N. Sumniangyen, P. Boontawan, M. Ketudat-Cairns, A. Boontawan & P. Boonchuen. 2023. Effect of fed dietary yeast (*Rhodotorula paludigena* CM33) on shrimp growth, gene expression, intestinal microbial, disease resistance, and meat composition of *Penaeus vannamei*. Developmental & Comparative Immunology. 147: 104896. https://doi.org/10.1016/j.dci.2023.104896

Srirengaraj, V., H.L. Razafindralambo, H.N. Rabetafika, H.-T. Nguyen & Y.-Z. Sun. 2023. Synbiotic agents and their active components for sustainable aquaculture: Concepts, action mechanisms, and applications. Biology. 12 (12): 1498. https://doi.org/10.3390/biology12121498

Supriatna, S., A. Darmawan & A. Maizar. 2023. Pathway analysis of pH in whiteleg shrimp, *Penaeus vannamei* concrete pond intensifies in Banyuwangi East Java. IOP Conference Series: Earth and Environmental Science. 1191 (1): 012015. https://doi.org/10.1088/1755-1315/1191/1/012015

Suryana, A., E.N.N. Asih & I. Insafitri. 2023. Phenomenon of acute hepatopancreatic necrosis disease infection in vaname shrimp cultivation in Bangkalan Regency. Journal of Marine Research. 12 (2): 212-220. https://doi.org/https://doi.org/10.14710/jmr.v12i2.35632

Tassanakajon, A., K. Somboonwiwat, P. Supungul & S. Tang. 2013. Discovery of immune molecules and their crucial functions in shrimp immunity. Fish & Shellfish Immunology, 34 (4): 954-967. https://doi.org/10.1016/j.fsi.2012.09.021

Tran, L., L. Nunan, R. Redman, L. Mohney, C. Pantoja, K. Fitzsimmons & D. Lightner. 2013. Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. Diseases of Aquatic Organisms. 105 (1): 45-55. https://doi.org/10.3354/dao02621

Urquhart, E.A., S.H. Jones, J.W. Yu, B.M. Schuster, A.L. Marcinkiewicz, C.A. Whistler & V.S. Cooper. 2016. Environmental conditions associated with elevated *Vibrio parahaemolyticus* concentrations in Great Bay Estuary, New Hampshire. PLOS ONE. 11 (5): e0155018. https://doi.org/10.1371/journal.pone.0155018

Verdegem, M., A.H. Buschmann, U.W. Latt, A.J.T. Dalsgaard & A. Lovatelli. 2023. The contribution of aquaculture systems to global aquaculture production. Journal of the World Aquaculture Society. 54 (2): 206-250. https://doi.org/10.1111/jwas.12963

Wang, C., D. Yao, M. Zhao, K. Lu, Z. Lin, X. Chen, Y. Zhao & Y. Zhang. 2022. Shrimp lipid droplet protein perilipin involves in the pathogenesis of AHPND-Causing *Vibrio parahaemolyticus*. International Journal of Molecular Sciences. 23 (18): 10520. https://doi.org/10.3390/ijms231810520

Wang, W., X. Wu, Z. Liu, H. Zheng & Y. Cheng. 2014. Insights into hepatopancreatic functions for nutrition metabolism and ovarian development in the crab *Portunus trituberculatus*: Gene discovery in the comparative transcriptome of different hepatopancreas stages. PLoS ONE. 9 (1): e84921. https://doi.org/10.1371/journal.pone.0084921

Widigdo, B., M. Yuhana, A. Iswantari, C. Madonsa, I.D. Sapitri, Y. Wardiatno, A.A. Hakim & F. Nazar. 2021. The impact of nitrifying probiotic to population growth of pathogenic bacteria, *Vibrio* sp., and toxic nitrogen gasses in marine shrimp culture media under laboratory condition. Jurnal Pengelolaan Sumberdaya Alam Dan Lingkungan (Journal of Natural Resources and Environmental Management). 11 (1): 130-140. https://doi.org/10.29244/jpsl.11.1.130-140

Wiradana, P.A., A.Y. Maharani, M.D. Sani, R.J.K. Susilo, M.I. Riandi, I.G. Widhiantara, P.E. Sudaryatma, T. Okabayashi & A.T. Mukti. 2023. Monitoring of microbial, water quality, and production performance of *Penaeus vannamei* on intensive pond in Bulukumba, South Sulawesi, Indonesia. IOP Conference Series: Earth and Environmental Science, 1273 (1): 012058. https://doi.org/10.1088/1755-1315/1273/1/012058

Wiradana, P.A., G. Mahasri, R.E.R. Sari, U.C. Marwiyah & R. Prihadhana. 2019. Identification of white spot syndrome virus (WSSV) in pacific white shrimps (*Penaeus vannamei*) from ponds postexposure to immunogenic membrane proteins (*Zoothamnium penaei*). IOP Conference Series: Earth and Environmental Science. 236 (1). https://doi.org/10.1088/1755-1315/236/1/012085

Wiradana, P.A., M.D. Sani, R.E. Mawli, F.N.D. Ashshoffa, I.G. Widhiantara & A.T. Mukti. 2022. Monitoring the occurrence of zoea syndrome (ZS) in pacific white shrimp (*Penaeus vannamei*) larval from several hatcheries in East Java, Indonesia. IOP Conference Series: Earth and Environmental Science. 1036 (1): 012003. https://doi.org/10.1088/1755-1315/1036/1/012003

Wiyoto, W., S. Sukenda, E. Harris, K. Nirmala & D. Djokosetiyanto. 2016. Water quality and sediment profile in shrimp culture with different sediment redox potential and stocking densities under laboratory condition. ILMU KELAUTAN: Indonesian Journal of Marine Sciences. 21 (2): 65. https://doi.org/10.14710/ik.ijms.21.2.65-76

Xu, C., E. Li, Y. Liu, S. Wang, X. Wang, K. Chen, J.G. Qin & L. Chen. 2018. Effect of dietary lipid level on growth, lipid metabolism and health status of the pacific white shrimp *Penaeus vannamei* at two salinities. Aquaculture Nutrition. 24 (1): 204-214. https://doi.org/10.1111/anu.12548

Yoshikawa, T., O. Murata, K. Furuya & M. Eguchi. 2007. Short-term covariation of dissolved oxygen and phytoplankton photosynthesis in a coastal fish aquaculture site. Estuarine, Coastal and Shelf Science. 74 (3): 515-527. https://doi.org/10.1016/j.ecss.2007.05.004

Yudiati, E., A. Isnansetyo, M. Murwantoko, A. Ayuningtyas, T. Triyanto & C.R. Handayani. 2016. Innate immune-stimulating and immune genes up-regulating activities of three types of alginate from Sargassum siliquosum in pacific white shrimp, *Penaeus vannamei*. Fish & Shellfish Immunology. 54: 46-53. https://doi.org/10.1016/j.fsi.2016.03.022

Yudiati, E., A. Isnansetyo, M. Murwantoko, T. Triyanto & C.R. Handayani. 2019. Alginate from *Sargassum siliquosum* simultaneously stimulates innate immunity, upregulates immune genes, and enhances resistance of pacific white shrimp (*Penaeus vannamei*) against white spot syndrome virus (WSSV). Marine Biotechnology. 21 (4): 503-514. https://doi.org/10.1007/s10126-019-09898-7

Zhang, H., Z. Sun, B. Liu, Y. Xuan, M. Jiang, Y. Pan, Y. Zhang, Y. Gong, X. Lu, D. Yu, D. Kumar, X. Hu, G. Cao, R. Xue & C. Gong. 2016. Dynamic changes of microbial communities in *Penaeus vannamei* cultures and the effects of environmental factors. Aquaculture. 455: 97-108. https://doi.org/10.1016/j.aquaculture.2016.01.011

Zhang, M., L. Pan, D. Fan, J. He, C. Su, S. Gao & M. Zhang. 2021. Study of fermented feed by mixed strains and their effects on the survival, growth, digestive enzyme activity and intestinal flora of *Penaeus vannamei*. Aquaculture. 530: 735703. https://doi.org/10.1016/j.aquaculture.2020.735703

Zheng, Z., R. Li, J.J. Aweya, D. Yao, F. Wang, S. Li, T.N. Tuan & Y. Zhang. 2021. The PirB toxin protein from *Vibrio parahaemolyticus* induces apoptosis in hemocytes of *Penaeus vannamei*. Virulence. 12 (1): 481-492. https://doi.org/10.1080/21505594.2021.1872171

Zokaeifar, H., J.L. Balcázar, C.R. Saad, M.S. Kamarudin, K. Sijam, A. Arshad & N. Nejat. 2012. Effects of *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, *Penaeus vannamei*. Fish & Shellfish Immunology. 33 (4): 683-689. https://doi.org/10.1016/j.fsi.2012.05.027