

Research Article

Encapsulated Freeze-Dried Lactic Acid Bacteria Enhance Immune Response and Support Growth of *Penaeus monodon* (Fabricius, 1798) in Hatchery and Earthen Pond Culture

Narongchai Chupoon^{*}, Nomchit Kaewthai Andre, Sirinat Srionnual and Thanikan Thorasin

Department of Food Innovation and Management, Faculty of Agro-Industry, Rajamangala University of Technology Srivijaya, Thung Yai, Nakhon Si Thammarat 80240, Thailand.

ABSTRACT

Article history:

Received: 2024-05-07

Revised: 2025-11-05

Accepted: 2025-11-27

Keywords:

Lactic acid bacteria (LAB);

Encapsulation;

Immune response;

Vibrio parahaemolyticus;

Shrimp aquaculture;

Microbiota profiling

This study investigated the effects of dietary supplementation with freeze-dried encapsulated *Lactobacillus* spp. (FEL) on the immune response, intestinal microbiota, and growth performance of black tiger shrimp (*Penaeus monodon*) under both hatchery and pond conditions. FEL, prepared with four lactic acid bacteria (LAB) strains and encapsulated in sodium alginate, was incorporated into diets at concentrations of 0%–1.0% (w/w). In hatchery trials, juvenile shrimp fed FEL-supplemented diets (0.2–1.0%) exhibited significantly enhanced total hemocyte count, phenoloxidase activity, and clearance of *Vibrio parahaemolyticus*, achieving 100% survival compared with $82.22 \pm 8.02\%$ in controls ($p < 0.001$). Shrimp fed 0.2% FEL also showed a significantly higher specific growth rate ($1.94\% \text{ day}^{-1}$) compared with controls ($1.33\% \text{ day}^{-1}$, $p < 0.05$). Moreover, FEL supplementation promoted LAB colonization and reduced intestinal *Vibrio* densities. In pond trials, shrimp were cultured for 100 days, with growth monitoring conducted from day 60 to day 100. Microbiota analysis by 16S rRNA gene sequencing revealed an increased abundance of beneficial Firmicutes and *Phaeobacter inhibens* and a decreased abundance of Proteobacteria and *Photobacterium damsela* in FEL-fed shrimp. Shrimp receiving 0.2% FEL supplementation also exhibited consistently greater body weight and length than the control group, with significant differences observed at later sampling points ($p < 0.05$). These findings demonstrate that FEL supplementation can enhance innate immunity, modulate the gut microbiota, and promote shrimp growth, highlighting its potential as a sustainable probiotic feed additive for shrimp aquaculture.

© 2026 Chupoon, N., Andre, N.K., Srionnual, S. and Thorasin, T. Recent Science and Technology published by Rajamangala University of Technology Srivijaya

1. Introduction

Thailand, once a leading global exporter of black tiger shrimp (*P. monodon*), has experienced a marked decline in production due to recurring disease outbreaks, particularly white spot syndrome virus (WSSV) and early mortality syndrome (EMS) (Han *et al.*, 2022; Leano and Mohan, 2020). These challenges highlight the urgent need for sustainable disease management strategies in shrimp aquaculture. Among various alternatives to antibiotics, probiotics have gained increasing attention due to their ability to enhance growth, improve immune responses, and reduce disease susceptibility (Hai, 2015; Hosein *et al.*, 2021; Kesarcodi-Watson *et al.*, 2012). Recent research indicates that

dietary probiotic supplementation modulates the shrimp gut microbiota, improving nutrient uptake and stimulating host immune functions (Chin *et al.*, 2024a; Lulijwa *et al.*, 2020).

Within the broad range of probiotic candidates, LAB are the most widely studied due to their antimicrobial properties and immunostimulatory effects, including the suppression of pathogenic *Vibrio* spp. (Zhou *et al.*, 2023; Huisakul *et al.*, 2007). However, the practical application of LAB faces challenges, particularly maintaining viability during feed processing and gastrointestinal passage.

To address this, microencapsulation using biopolymers such as sodium alginate has emerged as a promising approach,

^{*} Corresponding author.

E-mail address: narongchai.c@rmutsv.ac.th

Cite this article as:

Chupoon, N., Andre, N.K., Srionnual, S. and Thorasin, T. 2026. Encapsulated Freeze-Dried Lactic Acid Bacteria Enhance Immune Response and Support Growth of *Penaeus monodon* (Fabricius, 1798) in Hatchery and Earthen Pond Culture. *Recent Science and Technology* 18(1): e267534.

<https://doi.org/10.65411/rst.2026.267534>

improving probiotic stability, targeted release, and colonization in the intestinal tract (Afzaal *et al.*, 2020; Anal and Singh, 2021).

Despite these advances, most research on encapsulated probiotics has been confined to controlled laboratory settings. Field trials validating their effects in commercial aquaculture remain scarce, particularly when integrated with advanced microbiome profiling techniques. Addressing this gap is essential for translating laboratory success into practical implementation (Martínez-Porchas *et al.*, 2021; Fan *et al.*, 2023; Khanjani *et al.*, 2024; Vieira *et al.*, 2021).

Therefore, this study tested the hypothesis that dietary supplementation with FEL enhances the immune response, survival, growth, and gut microbiota stability of *P. monodon*. To evaluate this, experiments were conducted under both laboratory conditions, including pathogen challenge tests, and field conditions in earthen ponds. Practical application was assessed at a 0.2% FEL inclusion level, and gut microbiota dynamics were analyzed using 16S rRNA gene sequencing.

2. Materials and Methods

2.1 Experimental design overview

This study was conducted in two phases. Phase 1, conducted as a hatchery trial, employed a completely randomized design (CRD) with six dietary treatments of FEL ranging from 0 to 1.0% (w/w). Growth performance, survival during the feeding period, immune responses, and the intestinal LAB and *Vibrio* spp. were evaluated, along with resistance to *V. parahaemolyticus* challenge. Phase 2, a pond trial, validated the optimal FEL inclusion level of 0.2% identified in Phase 1. Two earthen ponds were used, each subdivided into cages, and shrimp were reared under farm-like conditions. Growth performance and intestinal microbiota composition were assessed, with statistical comparisons between the control and 0.2% FEL groups performed using independent-samples *t*-tests.

2.2 FEL preparation

This study utilized four strains of LAB: *Lactobacillus acidophilus* TISTR 236, *L. plantarum* TISTR 542, *L. casei* TISTR 389, and *L. bulgaricus* TISTR 451, obtained from the Thailand Institute of Scientific and Technological Research (TISTR, Thailand), for cell mass production and encapsulation. Pure freeze-dried cells of LAB activated by inoculating them into de Man, Rogosa and Sharpe (MRS) broth and incubating at 37 °C for 24 h. The fermentation process was conducted in a 5-L bioreactor (Bioflo III System, New Brunswick Scientific Co. Inc., NJ, USA) containing 3 L of sterilized MRS broth. After cooling, the activated inoculum was added. Each fermentation batch was inoculated with 5% (v/v) LAB culture, previously grown in MRS broth, at an initial concentration of approximately 1×10^5 CFU mL⁻¹.

Fermentation was maintained at 43 ± 2 °C with an agitation rate of 50 rpm. The pH was regulated at 5.0 throughout fermentation using a base pump with 4.0 mol L⁻¹ NaOH. The

process was carried out under anaerobic conditions for 48 h, after which the cell suspension was harvested for encapsulation. The harvested LAB suspension was co-encapsulated using sodium alginate microgels, following the method described by Afzaal *et al.* (2020). Briefly, the LAB culture suspension was mixed with a sodium alginate solution for 5 min to achieve a final concentration of 1×10^9 CFU mL⁻¹. The mixture was then injected into a 0.1 M calcium chloride hardening solution to form beads. The beads were collected by filtration, washed with sterile deionized water, and stored in physiological saline solution at 4 °C until freeze-drying.

The diameter of freshly prepared alginate gel beads was 2.41 ± 0.07 mm ($n = 10$; range: 2.3–2.5 mm). Size distribution analysis revealed that 20% of the beads were between 2.3–2.4 mm, 60% between 2.4–2.5 mm, and 20% between 2.5–2.6 mm, indicating a narrow and uniform distribution. Finally, the FEL powder was incorporated into the experimental diets, as described in Section 2.3.

2.3 Shrimp husbandry and feeding protocol

Two independent cohorts of *P. monodon* were used in this study: one cohort for the hatchery feeding and challenge trial (Phase 1) and a second cohort for the earthen-pond validation trial (Phase 2). *P. monodon* post-larvae (PL-5) were purchased from a certified pathogen-free hatchery in Ranod District, Songkhla Province, Thailand. Upon arrival, the post-larvae were acclimatized and reared in nursery tanks at the Aquaculture Research Facility, Faculty of Science and Fisheries Technology, Trang Campus, Rajamangala University of Technology Srivijaya (RUTS), Thailand. The shrimp were maintained under standard aquaculture conditions with continuous aeration and daily water-quality monitoring to ensure optimal rearing. During the nursery phase (PL-5 to PL-30), shrimp were fed a commercial starter diet formulated for early juvenile shrimp (Phoca Starter P5–P30; Phoca Feed Co., Ltd., Thailand) containing approximately 40–42% crude protein.

After the nursery stage, shrimp were transferred to 1,000-L fiberglass tanks equipped with a recirculating seawater system and continuous aeration (150 shrimp tank⁻¹). Animals were fed a basal Phoca Feed diet for 20 days, after which six experimental diets containing FEL at 0, 0.2, 0.4, 0.6, 0.8, or 1.0% (w/w) were administered following Zhang *et al.* (2018) and Argue *et al.* (2002). For FEL diet preparation, soybean oil (2% of feed weight) was used as a binder; FEL powder was top-coated onto finished pellets, gently tumbled to ensure uniform adhesion, and air-dried at ≤ 35 °C to restore surface dryness. Diets were broadcast at 5–6% of body weight per day in five equal feedings (06:00, 10:00, 14:00, 18:00, and 22:00 h), with three replicate tanks per treatment.

Proximate composition analyses were conducted in triplicate following AOAC (2016) standard methods for crude protein, lipid, fiber, moisture, and ash determinations. Nitrogen-free extract (NFE) was calculated by difference.

2.4 Challenge trials

At the end of the 50-day feeding period, juvenile *P. monodon* (mean weight 5.0 ± 0.20 g) from each dietary treatment were transferred to the Biotechnology Laboratory, Faculty of Agro-Industry, RUTS, Nakhon Si Thammarat Campus, Thailand, for the challenge trial. Upon arrival, shrimp were distributed into 100-L aquaria (15 shrimp per aquarium; three replicate aquaria per treatment) and acclimated for 10 days under standard laboratory conditions.

After acclimation, shrimp were challenged with *V. parahaemolyticus*. The bacterial strain was cultured in tryptic soy broth (TSB) supplemented with 1.5% NaCl at 30 °C for 18 h. The bacterial suspension was harvested by centrifugation, washed twice with sterile saline, and resuspended in 1.5% saline solution. The concentration was adjusted to 2×10^7 CFU mL⁻¹ using a spectrophotometer and confirmed by plate counts on thiosulfate citrate bile salts sucrose (TCBS) agar. Each shrimp was injected intramuscularly in the third abdominal segment with 0.1 mL of the bacterial suspension, whereas control shrimp were injected with sterile saline. Following injection, shrimp were maintained under the same conditions for 9 days, and mortality was recorded twice daily.

Colony counts were expressed as log₁₀ colony-forming units (CFU g⁻¹) of intestinal tissue. Homogenates were kept at 4 °C and processed within 24 h to maintain microbial viability. All procedures were performed aseptically to preserve sample integrity.

2.5 Sampling and analyses

Samples of hemolymph and intestinal tissue were collected in triplicate from challenged shrimp on day 9 at the time points described in Section 2.4. These samples were subjected to immunological, microbiological, and growth performance analyses. Total hemocyte count (THC), clearance ability (CA), and phenoloxidase (PO) activity were determined to assess the shrimp's immune response to *V. parahaemolyticus* challenge, while intestinal microbiological parameters and growth performance were also evaluated.

2.5.1 Immune response

Hemolymph samples were randomly collected on day 9 of the challenge for immune analyses. THC, CA, and PO activity were measured as described below.

THC was measured using a hemocytometer under a light microscope. Hemolymph was diluted 1:1 with an anticoagulant solution, and hemocyte counts were performed in triplicate to ensure accuracy. The mean cell count for each sample was calculated and expressed as cells mL⁻¹ of hemolymph.

The CA of *V. parahaemolyticus* in the hemolymph of *P. monodon* was evaluated following a modified method of van de Braak *et al.* (2002). Shrimp were injected intramuscularly in the third abdominal segment with 100 µL of a *V. parahaemolyticus*

suspension ($\approx 1 \times 10^5$ CFU mL⁻¹) prepared in sterile 0.9% (w/v) saline. Hemolymph was collected from the ventral sinus at 0 and 3 h post-injection using a sterile syringe containing anticoagulant solution (10 mM sodium citrate, 450 mM NaCl, pH 7.3). Serial dilutions were plated on TCBS and incubated at 37 °C for 24 h. Bacterial densities were expressed as log₁₀ CFU mL⁻¹ of hemolymph. CA was calculated according to Equation (1):

$$CA (\%) = \frac{(N_0 - N_t)}{N_0} \times 100 \quad (1)$$

where N_0 and N_t are bacterial counts (CFU mL⁻¹) in hemolymph at 0 h and 3 h post-injection, respectively.

PO activity was assayed using L-DOPA as substrate. Hemolymph was centrifuged at $3,000 \times g$ for 10 min to obtain cell-free plasma, which was incubated with 10 mM L-DOPA at 28 °C. Optical density at 490 nm was recorded every minute for 10 min, and PO activity expressed as the rate of absorbance change (OD₄₉₀ min⁻¹).

2.5.2 Microbiological analysis and enumeration of LAB and *Vibrio* spp.

Intestinal samples were collected from juvenile *P. monodon* (≈ 69 days old; mean weight 6.26 ± 0.21 g) at the end of the *V. parahaemolyticus* challenge trial (Day 9) to quantify LAB and *Vibrio* spp. Shrimp were euthanized in ice-cold seawater, and intestines were aseptically dissected using sterile scissors and forceps.

For each dietary treatment (FEL 0, 0.2, 0.4, 0.6, 0.8, and 1.0% w/w), three biological replicates were prepared by pooling intestines from 4–6 shrimp to obtain approximately 0.20 g wet tissue per replicate. Pooled tissues were placed in sterile 2 mL tubes containing 1.8 mL of sterile 0.85% NaCl to yield a 1:10 (w/v) homogenate and homogenized with a stomacher. Serial ten-fold dilutions were plated on TCBS agar for enumeration of *Vibrio* spp. and on MRS agar for LAB. TCBS plates were incubated at 37 °C for 24 h, and MRS plates at 37 °C for 24–48 h under anaerobic conditions.

Colony counts were expressed as log₁₀ colony-forming units (CFU g⁻¹) of intestinal tissue. Homogenates were kept at 4 °C and processed within 24 h to maintain microbial viability. All procedures were performed aseptically to preserve sample integrity.

2.5.2.1 Intestinal microbiota profiling

For pond-reared shrimp, the intestinal microbial community was profiled using 16S rRNA gene amplicon sequencing. Shrimp were reared in three replicate cages per dietary treatment (≈ 200 individuals per cage). For each treatment, intestines from five shrimp per cage were pooled to obtain approximately 100 mg of wet tissue, yielding three biological replicates per treatment (control and 0.2% FEL).

All dissections were performed aseptically on ice. Composite intestinal samples were immediately placed in 2 mL screw-cap tubes containing zirconia/silica beads, snap-frozen in liquid nitrogen, and stored at -80 °C until DNA extraction.

DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany) and quantified with a NanoDrop spectrophotometer (NanoDrop Technologies, USA). The V3–V4 hypervariable regions of the 16S rRNA gene were amplified by polymerase chain reaction (PCR) using Illumina overhang adapter primers and Phusion High-Fidelity DNA polymerase (Bio-Rad, UK) under the following thermal cycling conditions: initial denaturation at 95 °C for 3 min; 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s; followed by a final extension at 72 °C for 5 min.

Amplicons were purified with AMPure XP magnetic beads (Beckman Coulter, USA), indexed using the Nextera XT Index Kit v2 (Illumina, USA), and pooled at equimolar concentrations. The pooled library was spiked with 5% PhiX control and sequenced on an Illumina MiSeq platform (2 × 300 bp paired-end reads) using the MiSeq Reagent Kit v3 (600-cycle). Raw sequence data were processed using MiSeq Reporter Software (Illumina, USA).

2.5.3 Growth performance evaluation

Following a 10-day acclimation period and a 50-day feeding phase, shrimp were subjected to a 9-day *Vibrio* challenge test. After the challenge, shrimp were weighed to determine specific growth rate (SGR) and survival rate. The calculations were performed using the following formulas, as described by Soundarapandian and Velmurugan (2008).

The SGR was calculated as:

$$\text{SGR (\% day}^{-1}\text{)} = \frac{(\ln W_t - \ln W_0)}{t} \times 100 \quad (2)$$

where W_t is the final mean weight (g), W_0 is the initial mean weight (g), and t is the rearing period (days).

2.6 Field trial methods

The pond trial (Phase 2) was conducted at the shrimp-farming facility of the Faculty of Science and Fisheries Technology, RUTS, Trang Province, Thailand (Mai Fad Subdistrict, Sikao District; 7.5186 °N, 99.2682 °E). Two earthen ponds (1,600 m²; 1.8 m depth) were used: one served as the control (standard diet) and the other received the diet supplemented with 0.2% FEL. Each pond contained three replicate net cages (4 × 4 m; ≈ 6–7 shrimp m⁻³) and was continuously aerated by four 0.75-kW paddlewheel aerators paddlewheel to maintain dissolved oxygen above 5 mg L⁻¹.

Shrimp stocking and transfer procedures followed the protocol described in Section 2.3, including the nursery phase and acclimation. Fifty-day-old shrimp (initial mean weight = 4.7 ± 0.2 g, mean ± SD) were acclimated in the ponds for 10 days

prior to the start of the 40-day feeding period (days 60–100 of the culture cycle). At day 60, the control pond received the standard diet, whereas the treatment pond received the diet supplemented with 0.2% FEL.

Growth performance was monitored on days 60, 70, 80, 90, and 100. At each sampling point, ten shrimp were randomly collected from each cage to determine body weight (± 0.5 g; Sartorius Praxium 2102-1S, Germany) and total length (± 0.1 cm) using a digital caliper. SGR was calculated as described in Section 2.5.3. At the end of the 100-day culture period, intestinal samples were collected for microbiota profiling as outlined in Section 2.5.2.1.

2.6.1 Water quality monitoring

Water quality was monitored daily throughout the trial. Salinity was maintained at 15–20 ppt by regular exchange with brackish water and measured with a Salinity Refractometer (MASTER-S/MillM, Atago, Japan). Dissolved oxygen (DO) and pH were measured using a multi-parameter water-quality meter (HI9829; pH/ISE/EC/DO/Turbidity, Hanna Instruments, USA), with DO kept above 5 mg L⁻¹ and pH maintained between 7.5 and 8.5. Nitrite–nitrogen was kept below 0.1 mg L⁻¹ and analyzed using a Hach NI-12 Nitrate–Nitrite Water Test Kit. Temperature was recorded with a digital thermometer.

All experimental procedures involving shrimp were conducted in accordance with the institutional guidelines for the care and use of aquatic animals of RUTS.

2.7 Statistical analysis

The hatchery trial (Phase 1) followed a CRD with six dietary treatments (0–1.0% FEL, w/w) and three replicates each. Data were analyzed by one-way ANOVA, and means were compared using Tukey's HSD ($p < 0.05$). For the pond trial (Phase 2), growth and microbiota data were compared between the control and 0.2% FEL groups using an independent-samples t -test ($p < 0.05$).

3. Results and Discussion

3.1 Proximate composition of experimental diets

The proximate composition of shrimp diets supplemented with different levels FEL is summarized in Table 1. Crude protein, lipid, fiber, ash, NFE, and moisture contents were comparable among all treatments, with crude protein ranging from 45.2 to 45.7%, lipid from 7.1 to 7.4%, and moisture from 11.2 to 11.9%. Negligible variations indicate that FEL inclusion up to 1.0% (w/w) did not affect the basic nutritional composition of the diets.

3.2 Immune enhancement in shrimp challenged with *V. parahaemolyticus*

As shown in Table 2, shrimp fed 0.2–1.0% (w/w) FEL exhibited significantly higher THC, CA, and PO activities than the control group. THC peaked in the 1.0% FEL treatment at

$10.10 \pm 0.25 \times 10^6$ cells mL^{-1} , compared with $4.97 \pm 0.09 \times 10^6$ cells mL^{-1} in controls ($p < 0.001$, $n = 3$). CA increased dose-dependently, reaching $22.53 \pm 0.66\%$ at 1.0% FEL ($p < 0.001$, $n = 3$), indicating a strong enhancement in cellular immune response. PO activity rose to 0.43 ± 0.009 OD min^{-1} at 0.8 % FEL ($p < 0.01$, $n = 3$) and remained elevated at 0.42 ± 0.018 OD min^{-1} in the 1.0% group ($p < 0.05$, $n = 3$). Dose-dependent increases in THC, CA, and PO indicate robust activation of cellular and enzymatic immune defenses, with PO activity plateauing beyond 0.8%, suggesting this level as the optimal immunostimulatory dose. These results are consistent with previous studies demonstrating that probiotic supplementation enhances immune response and resistance to *Vibrio* infection in *P. monodon* (Zokaeifar *et al.*, 2012).

The observed responses agree with previous reports that LAB enhance shrimp immunity by stimulating hemocyte production and the prophenoloxidase cascade (Nguyen *et al.*, 2025 b; Kongnum and Hongpattarakere, 2012 a; Feng *et al.*, 2020). Similar effects were observed by Zheng *et al.* (2017) in Pacific white shrimp (*Litopenaeus vannamei*), where dietary probiotics significantly reduced intestinal *Vibrio* counts, supporting the application of FEL as a functional feed additive for disease-resilient aquaculture.

3.3 Effect of FEL supplementation on intestinal LAB and *Vibrio* spp.

FEL supplementation significantly influenced the intestinal microbiota of *P. monodon* during the *V. parahaemolyticus*

challenge. As illustrated in Figure 1, shrimp fed FEL diets at 0.2–1.0% (w/w) exhibited a clear, dose-dependent decline in intestinal *Vibrio* density, with mean counts decreasing from 5.2 ± 0.4 log CFU g^{-1} in the control group to 1.1 ± 0.2 log CFU g^{-1} in shrimp receiving 0.8% FEL ($p < 0.001$). Conversely, the results presented in Figure 2 show that total LAB populations increased significantly across all FEL treatments ($p < 0.001$), reaching the highest density in the 1.0% group. These quantitative shifts confirm successful colonization of beneficial LAB while suppressing opportunistic *Vibrio* spp., demonstrating that FEL supports a more stable and health-promoting intestinal community.

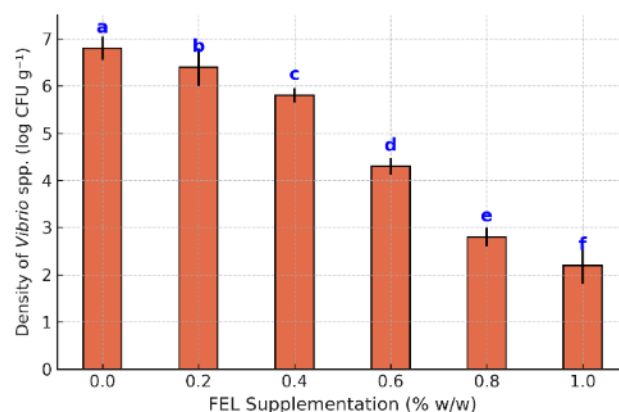


Figure 1 Density of intestinal *Vibrio* spp. in *P. monodon* fed FEL-supplemented diets after *V. parahaemolyticus* challenge. Note. Different letters indicate significant differences ($p < 0.001$).

Table 1 Proximate composition of shrimp diets supplemented with FEL (0–1.0% w/w).

FEL (% w/w)	Crude protein (%)	Crude lipid (%)	Crude fiber (%)	Ash (%)	NFE (%)	Moisture (%)
0.0	45.2 ± 0.27	7.2 ± 0.20	3.8 ± 0.14	9.6 ± 0.15	22.4 ± 0.65	11.8 ± 0.11
0.2	45.2 ± 0.23	7.2 ± 0.17	3.7 ± 0.11	9.3 ± 0.15	22.6 ± 0.43	11.8 ± 0.14
0.4	45.2 ± 0.22	7.1 ± 0.15	3.9 ± 0.14	9.0 ± 0.16	22.7 ± 0.45	11.7 ± 0.14
0.6	45.3 ± 0.26	7.3 ± 0.14	3.4 ± 0.17	9.0 ± 0.18	22.5 ± 0.38	11.9 ± 0.15
0.8	45.5 ± 0.27	7.2 ± 0.13	3.6 ± 0.12	9.4 ± 0.17	22.3 ± 0.41	11.2 ± 0.14
1.0	45.7 ± 0.24	7.4 ± 0.17	3.6 ± 0.12	9.0 ± 0.16	22.1 ± 0.60	11.2 ± 0.13

Note. Values are expressed as mean ± standard deviation ($n = 3$).

Table 2 Immune responses of *P. monodon* fed 0–1.0% FEL diets after 9 days of *V. parahaemolyticus* challenge.

FEL Level (%)	THC ($\times 10^6$ cells mL^{-1})	CA (%)	PO activity (OD ₄₉₀ min^{-1})
0	4.97 ± 0.09 ^d	2.67 ± 0.55 ^d	0.06 ^d
0.2	7.53 ± 0.32 ^c	4.37 ± 0.84 ^{cd}	0.26 ^c
0.4	8.80 ± 0.34 ^{bc}	13.97 ± 0.47 ^{bc}	0.31 ^c
0.6	10.00 ± 0.50 ^{ab}	15.30 ± 0.93 ^b	0.37 ^b
0.8	9.73 ± 0.41 ^{ab}	21.33 ± 0.58 ^a	0.43 ^a
1.0	10.10 ± 0.25 ^a	22.53 ± 0.66 ^a	0.42 ^a

Note. Values are expressed as mean ± SE ($n = 3$). Different superscript letters within columns indicate significant differences among treatments ($p < 0.05$).

The pronounced reduction in *Vibrio* coupled with enrichment of LAB suggests that encapsulation protected probiotic viability during feed processing and gastrointestinal transit, enabling efficient colonization and competitive exclusion of pathogens. A stable and dominant LAB population enhances nutrient

absorption and strengthens host resistance by producing antimicrobial metabolites and modulating intestinal pH. Such mechanisms are critical for maintaining microbial balance and host resilience under disease pressure.

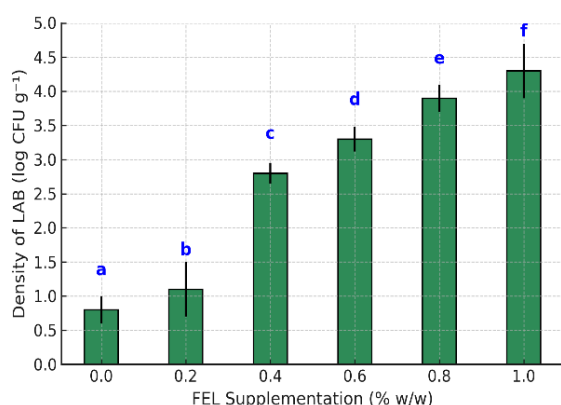


Figure 2 Density of intestinal LAB in *P. monodon* fed FEL-supplemented diets after *V. parahaemolyticus* challenge.

Note. Different letters indicate significant differences ($p < 0.001$).

Our results corroborate previous studies showing that LAB inhibit Gram-negative pathogens through organic acid production and immune stimulation (Ringø *et al.*, 2020; Vieira *et al.*, 2021). Comparable reductions in *Vibrio* loads and increases in LAB abundance have been reported in shrimp and other aquaculture species supplemented with encapsulated probiotics (Zheng *et al.*, 2017; Kim *et al.*, 2022), supporting FEL's potential as a functional microbial modulator in intensive shrimp farming.

The stability of LAB populations observed in FEL-treated groups during *V. parahaemolyticus* infection indicates enhanced probiotic colonization and survivability, likely attributed to the encapsulated form of the probiotics. Encapsulation has been reported to protect LAB from harsh gastrointestinal conditions, enabling them to reach the distal gut and establish more effectively (Vieira *et al.*, 2021). Such gut microbiota modulation improves intestinal health and may also enhance systemic immunity, including phagocytic activity and disease resistance.

Therefore, FEL supplementation represents a promising strategy for microbiota management in shrimp aquaculture, offering both preventive and stabilizing effects on beneficial intestinal bacteria, while simultaneously suppressing opportunistic pathogens such as *V. parahaemolyticus*.

3.4 Effects of FEL on growth and survival under pathogen challenge

The findings confirm that dietary supplementation with FEL significantly enhanced both growth performance and survival of *P. monodon* under *V. parahaemolyticus* challenge. SGR was markedly improved in all FEL-supplemented groups compared with the control ($p < 0.05$), with the highest value observed in the 0.2% FEL group ($1.94 \pm 0.01\% \text{ day}^{-1}$), whereas the control group exhibited the lowest growth ($1.33 \pm 0.06\% \text{ day}^{-1}$). These results align with previous studies reporting that probiotic supplementation can elevate SGR in shrimp under pathogenic stress (Nguyen *et al.*, 2025b; Zheng *et al.*, 2017; Kim *et al.*, 2022).

FEL supplementation likely promoted intestinal colonization, enzyme activity, and nutrient assimilation, thereby contributing to improved physiological resilience. Encapsulation may have protected probiotic viability during digestion, enabling effective

gut colonization. All FEL-treated groups achieved 100% survival, which was significantly higher than the control ($82.22 \pm 8.02\%$), indicating strong immunomodulatory effects. Previous studies have shown that dietary lactic acid bacteria can enhance immune responses and improve disease resistance in *P. monodon* (Nguyen *et al.*, 2019). These improvements suggest that FEL helps maintain gut health under pathogen stress. Hemocytes play a key role in bacterial clearance through phagocytosis and encapsulation, particularly against *Vibrio* spp., which may explain the improved survival observed in this study (van de Braak *et al.*, 2002).

Overall, FEL supplementation at 0.2–1.0% enhanced both growth and disease resistance, with 0.2% being the most effective for growth, as summarized in Table 3. These findings support the application of FEL as a practical dietary additive to improve shrimp performance and resilience in commercial aquaculture.

Table 3 SGR and survival of *P. monodon* at 9 days after *V. parahaemolyticus* challenge under FEL-supplemented diets.

FEL Level (%)	Mean SGR (% day ⁻¹)	Survival Rate (%)
0	1.33 ± 0.06^b	82.22 ± 8.02^b
0.2	1.94 ± 0.01^a	100.00 ± 0.00^a
0.4	1.92 ± 0.01^a	100.00 ± 0.00^a
0.6	1.89 ± 0.02^a	100.00 ± 0.00^a
0.8	1.84 ± 0.01^{ab}	100.00 ± 0.00^a
1.0	1.79 ± 0.02^b	100.00 ± 0.00^a

Note. Values are expressed as mean $\pm$ SE ($n = 3$). Different superscript letters within columns indicate significant differences among treatments ($p < 0.05$ for SGR; $p < 0.001$ for survival rate).

Survival analysis confirmed the protective effect of FEL supplementation, with all treated groups exhibiting 100% survival at 9 days post-*V. parahaemolyticus* challenge, compared with $82.22 \pm 8.02\%$ in the control group. This marked difference is attributed to the synergistic action of four *Lactobacillus* spp. in FEL, which not only function as probiotics but also produce extracellular polymeric substances (EPSs) with prebiotic-like properties. EPSs can enhance host immunity and suppress pathogen colonization in the shrimp gut. Nguyen *et al.* (2025a) reported that EPSs derived from *L. plantarum* and *Bifidobacterium bifidum* improved gut microbiota balance, reduced *V. parahaemolyticus* levels, and increased immune responses, including THC and PO activity. These effects likely contributed to the complete protection observed in FEL-fed shrimp.

Moreover, recent studies highlight the synergistic benefits of synbiotic approaches. For example, Khanjani *et al.* (2024) reported improved immunity and survival in *P. vannamei* fed synbiotic diets, while Nguyen *et al.* (2025a) also demonstrated enhanced immune responses and resistance to *V. parahaemolyticus* in shrimp supplemented with EPS derived

from LAB. Taken together, these findings suggest that the protective effects of FEL may be associated with EPS-mediated mechanisms reported in other studies (Nguyen *et al.*, 2025a), though EPS was not directly quantified in this study. The incorporation of such functional additives represents a sustainable alternative to antibiotics in intensive shrimp aquaculture.

Interestingly, no mortality was observed in any FEL-supplemented groups with 0.2–1.0% w/w following *V. parahaemolyticus* challenge. Although this clearly demonstrates the protective efficacy of FEL, the observed survival may also be influenced by the virulence characteristics of the bacterial strain employed. The *V. parahaemolyticus* isolate in this study is an AHPND-associated strain that primarily targets the hepatopancreas and induces acute tissue damage (Lai *et al.*, 2015). The absence of mortality in FEL-fed shrimp suggests that FEL supplementation may effectively mitigate the pathogenic effects of AHPND-related strains through enhanced gut immunity and beneficial microbial modulation. Nevertheless, further studies using multiple *V. parahaemolyticus* strains with differing virulence profiles are recommended to more comprehensively determine the extent of FEL's protective capabilities.

3.5 Evaluation of 0.2% FEL supplementation on growth performance of *P. monodon* in earthen pond culture

The growth trajectories of *P. monodon* in the pond trial are presented in Figure 3, showing that shrimp fed 0.2% FEL consistently exhibited greater body weight than the control group, particularly during the latter part of the 100-day culture period. These weight trajectories formed the basis for calculating the SGR during the 40-day monitoring period (days 60–100). The calculated SGR was $4.16 \pm 0.031\%$ day⁻¹ in the control group and $4.48 \pm 0.015\%$ day⁻¹ in the 0.2% FEL-supplemented group, representing a statistically significant improvement ($p < 0.001$).

Although the absolute increase was moderate, this enhancement is biologically meaningful and consistent with previous reports on *P. monodon* and related penaeid shrimp cultured under semi-intensive pond conditions, where SGR typically ranges from 3.5–4.5% day⁻¹ depending on initial size and culture conditions (Fan *et al.*, 2023; Nguyen *et al.*, 2025b; Sung *et al.*, 2021). Importantly, the growth-promoting effect observed in the FEL group ($\approx 0.3\%$ increase in SGR) is comparable to outcomes reported for dietary probiotics and microbial-derived feed additives, where improvements of 0.1–0.4% day⁻¹ are common (Tran *et al.*, 2023). This alignment with the broader literature strengthens the evidence that FEL can function as a practical functional additive in shrimp farming.

As illustrated in Figure 4, in addition to body weight, shrimp fed with 0.2% FEL also exhibited significantly greater body length compared to the control group at the end of the pond trial. This result further supports the growth-promoting effect of FEL under practical culture conditions. Moreover, shrimp fed

with 0.2% FEL also exhibited significantly greater body length at multiple time points, particularly on days 70, 80, and 100. These improvements in length growth align with the enhanced body weight observed in the same treatment group, suggesting that FEL supplementation supports balanced somatic growth by promoting both weight and length gain. The consistency of these trends across both parameters suggests that FEL may promote balanced growth by improving nutrient uptake, stimulating digestive efficiency, and enhancing gut microbial stability.

The selection of 0.2% FEL was based on both its demonstrated biological efficacy and economic feasibility, with an estimated production cost of approximately 1,000 THB per kilogram. Although the improvement in SGR was modest, the findings support the practical application of FEL as a functional feed additive in shrimp aquaculture. To extend its applicability, long-term studies under commercial farming conditions should be undertaken to confirm its cost-effectiveness and scalability.

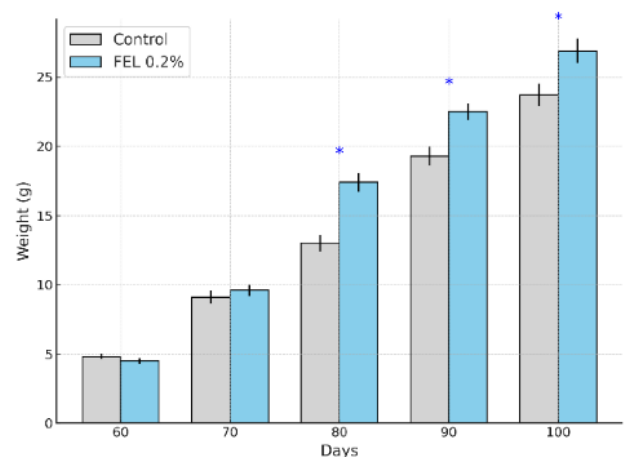


Figure 3 Effects of dietary 0.2% FEL supplementation on the body weight of *P. monodon* cultured in earthen ponds from days 60 to 100.

Note. Values are mean $\pm$ SD ($n = 10$). Asterisks (*) indicate significant differences between groups at each sampling point ($p < 0.05$, t -test).

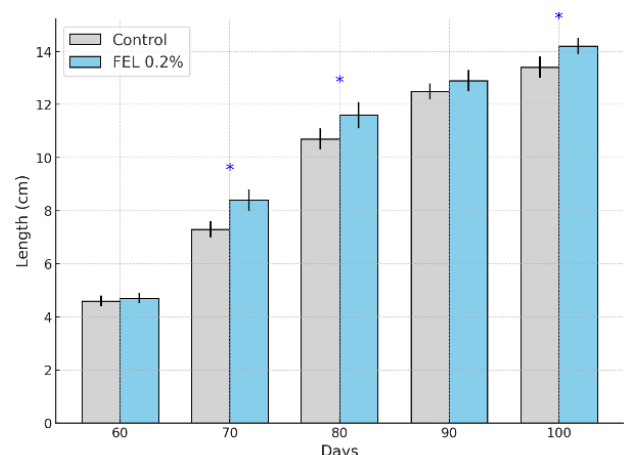


Figure 4 Effects of dietary 0.2% FEL supplementation on the body length of *P. monodon* cultured in earthen ponds from days 60 to 100.

Note. Values are mean $\pm$ SD ($n = 10$). Asterisks (*) indicate significant differences between groups at each sampling point ($p < 0.05$, t -test).

Visual observation indicated that shrimp in both control and FEL-supplemented groups exhibited comparable physical robustness and activity levels throughout the culture period. At harvest, the body weight of shrimp in both groups met the commercial market standard, indicating that FEL supplementation did not negatively affect shrimp morphology or growth uniformity. These findings support the practical applicability of 0.2% FEL in field conditions without compromising product quality or marketability.

Similar effects were reported by Sung *et al.* (2021), who demonstrated that dietary administration of LAB enhanced disease resistance and improved growth performance in *Litopenaeus vannamei* through modulation of immune parameters and intestinal microbiota. Likewise, Kongnum and Hongpattarakere (2012b) observed improved survival and growth in *P. monodon* fed with *L. plantarum*, underscoring the role of gut probiotics in promoting feed efficiency and physiological resilience. More recent studies (Li *et al.*, 2022; Tran *et al.*, 2023) further confirm that encapsulated probiotics enhance growth performance and gut health under both laboratory and field conditions.

Despite the positive outcomes observed, the limited pond replication warrants caution in interpretation. Future studies involving multiple farms and production cycles are recommended to substantiate these results.

3.6 Intestinal microbiota composition

3.6.1 Phylum-level analysis

Figure 5 presents the results of 16S rRNA sequencing at the phylum level, revealing that the intestinal microbiota of *P. monodon* was notably altered following supplementation with 0.2% FEL. Specifically, the relative abundance of Proteobacteria a phylum often associated with pathogenic bacteria and gut dysbiosis was significantly reduced in the FEL group compared to the control ($p < 0.05$). Conversely, the proportions of Actinobacteria and Firmicutes, both of which are commonly linked with beneficial functions such as immune modulation and gut barrier enhancement, were significantly increased ($p < 0.05$).

The observed changes in the gut microbiota suggest that FEL supplementation contributes to a more balanced and health-promoting intestinal ecosystem. Similar findings have been reported by Chin *et al.* (2024b) and Xiong (2024), who demonstrated that dietary probiotics can favorably modulate the intestinal microbiome of shrimp. Moreover, Gao *et al.* (2021) highlighted that an increase in Firmicutes abundance was associated with improved feed conversion efficiency and gut integrity in *L. vannamei*, while Nguyen *et al.* (2025b) showed that reducing *Proteobacteria* abundance via probiotic intervention could significantly lower the incidence of *Vibrio*-related infections.

This evidence is further supported by the work of Vieira *et al.* (2021) and Fan *et al.* (2023), who linked increased Firmicutes and Actinobacteria with enhanced immunity and

digestive efficiency. Likewise, the decline in Proteobacteria is consistent with previous studies showing that probiotic-driven modulation can suppress pathogenic taxa such as *Vibrio* spp. (Nguyen *et al.*, 2025b; Vega-Carranza *et al.*, 2024), underscoring the functional role of FEL in promoting disease resilience in shrimp.

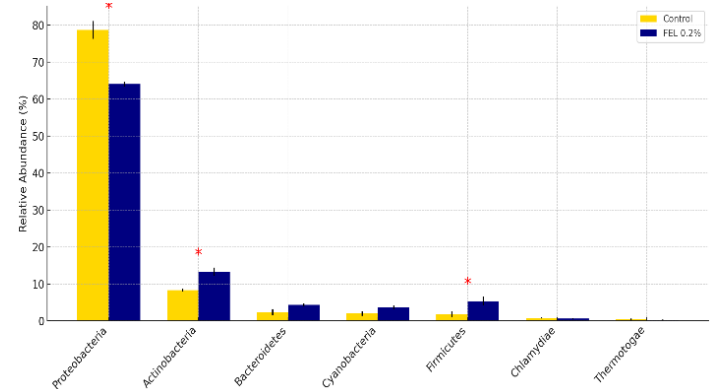


Figure 5 Phylum-level intestinal microbiota of *P. monodon* fed 0.2% FEL versus the control diet, based on 16S rRNA sequencing analysis (Illumina MiSeq).

Note. Data are presented as mean $\pm$ SD from three replicates. Asterisks (*) indicate phyla with significant differences between FEL and control groups ($p < 0.05$, *t*-test).

3.6.2 Species-level analysis

As depicted in Figure 6, at the species level, supplementation with 0.2% FEL significantly reduced the relative abundance of *P. damsela* ($p < 0.05$), a known shrimp pathogen, while increasing *P. inhibens* ($p < 0.05$). Notably, *P. inhibens* is a commensal species associated with enhanced immune regulation and pathogen suppression, which may contribute to improved disease resistance. This targeted microbial modulation suggests that FEL helps suppress harmful bacteria while promoting beneficial taxa that support intestinal resilience. These findings align with previous studies reporting the probiotic benefits of encapsulated *Bacillus* and *Lactobacillus* spp. in enhancing shrimp immunity and intestinal health (Zhou *et al.*, 2023; Ringø *et al.*, 2020; Sirikharin *et al.*, 2022).

The specific effect observed in *P. inhibens* underscores the importance of precision-targeted probiotics in modulating shrimp gut microbiota (Khanjani *et al.*, 2024). Future studies should investigate the functional genomics and metabolomic interactions of dominant commensal species to clarify their roles in host immunity and performance. Additionally, long-term trials under commercial farming conditions are recommended to validate FEL's efficacy and microbial stability over extended culture periods (Tran *et al.*, 2023).

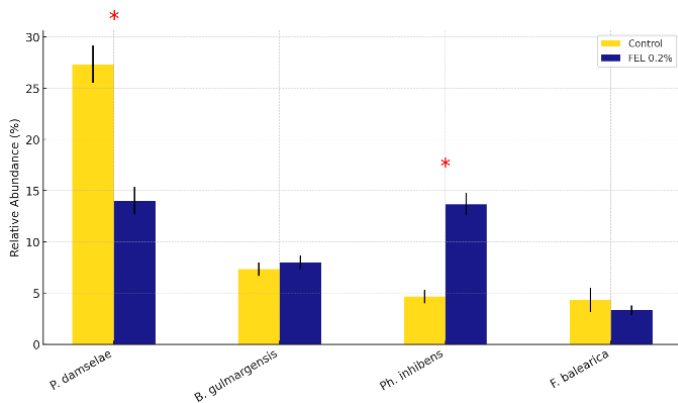


Figure 6 Relative abundance (%) of selected intestinal bacterial species in *P. monodon* fed the control and 0.2% FEL diets.

Note. Data are presented as mean $\pm$ SD from three replicates. Asterisks (*) indicate species with significant differences between FEL and control groups ($p < 0.05$, t -test). *P. damsela* (*Photobacterium damsela*), *B. gulgargensis* (*Blastochloris gulgargensis*), *Ph. inhibens* (*Phaeobacter inhibens*), *F. balearica* (*Ferrimonas balearica*).

The enrichment of *P. inhibens* may also improve mucosal immunity and microbial competition, creating a more stable gut environment. The use of next-generation sequencing (NGS) enabled precise detection of both dominant and rare taxa, facilitating deeper insight into host–microbiota interactions and supporting the development of targeted probiotic strategies in shrimp aquaculture. The ability of FEL to selectively enrich beneficial species like *P. inhibens* while suppressing pathogens such as *P. damsela* demonstrates its potential as a precision microbiome modulator.

This targeted effect may result from enhanced probiotic viability and delivery efficiency provided by encapsulation technology (Andriani *et al.*, 2022; Anal and Singh, 2021). Unclassified bacterial taxa detected in this study further emphasize the need for metagenomic and functional characterization to uncover the roles of currently unidentified microorganisms in shrimp gut ecology. Continued research into species-level interactions, including gene expression and metabolite production, will advance our understanding of FEL's long-term benefits and facilitate its application in commercial aquaculture (Khanjani *et al.*, 2024; Tran *et al.*, 2023).

While these findings are promising, they are based on observations from only two ponds with three replicate cages and should therefore be interpreted with caution.

4. Conclusion

Dietary supplementation with FEL enhanced immune responses, reduced intestinal *Vibrio* loads, and improved the growth performance of *P. monodon* under both laboratory and preliminary field conditions. FEL also modulated the intestinal microbiota by increasing beneficial Firmicutes and decreasing pathogenic taxa, underscoring its potential as a cost-effective and sustainable feed additive for shrimp health and productivity.

Further research should include larger-scale and multi-site trials across diverse aquaculture environments to validate its effectiveness under commercial conditions and to evaluate its safety, effects on meat quality, and economic feasibility for large-scale application.

5. Acknowledgments

The authors wish to express their sincere gratitude to the National Research Council of Thailand (NRCT) for funding this research under the fiscal year 2017 budget. Special thanks are also extended to the Faculty of Science and Technology of Fisheries, RUTS, Trang Campus, for providing access to the aquaculture laboratory and scientific equipment necessary for conducting this research. The authors would also like to thank the Office of Scientific Instrument and Testing, Prince of Songkla University, for performing the NGS analysis of the microbial community.

6. References

- Afzaal, M., Saeed, F., Hussain, S., Mohamed, A.A., Alamri, M. S., Ahmad, A., Ateeq, H., Tufail, T. and Hussain, M. 2020. Survival and storage stability of encapsulated probiotic under simulated digestion conditions and on dried apple snacks. **Food Science & Nutrition** 8(5): 5392-5401.
- AOAC. 2016. **Official Methods of Analysis, 20th ed.** AOAC International, Gaithersburg, MD, USA.
- Anal, A.K. and Singh, H. 2021. Recent advances in microencapsulation of probiotics for applications in functional foods: A review. **Critical Reviews in Food Science and Nutrition** 61(5): 748-763.
- Andriani, Y., Widanarni, W. and Ekasari, J. 2022. Encapsulation of probiotics for improved stability and efficacy in aquaculture: A review. **Aquaculture Reports** 24: 101088.
- Argue, B.J., Arce, S.M., Lotz, J.M. and Moss, S.M. 2002. Selective breeding of Pacific white shrimp (*Litopenaeus vannamei*) for growth and resistance to Taura Syndrome Virus. **Aquaculture** 204(3-4): 447-460.
- Chin, Y.K., Chin, Y.K., Haifa-Haryani, W.O., Nazarudin, M.F., Ahmad, M.I., Azzam-Sayuti, M., Mohamad, A., Ida-Muryany, M.Y., Karim, M.M., Annas, S., Azmai, M.N.A., Amal, M.N.A., Mohd Nor, N. and Ina-Salwany, M. 2024a. The synergistic *Lactobacillus plantarum* L20 and *Sargassum polycystum*-added diet for improvement of black tiger shrimp *Penaeus monodon*'s growth, immune responses, bacterial profiles, and resistance against *Vibrio harveyi*. **Aquaculture Reports** 34: 101903.
- Chin, Y.Y., Liew, H.C. and Ooi, P.T. 2024b. Dietary supplementation with *Lactobacillus plantarum* L20 and *Sargassum polycystum* enhances shrimp gut microbiota and disease resistance. **Aquaculture Reports** 29: 101601.

- Fan, Y., Zhang, H., Liu, W., Zhang, Y. and Wang, W. 2023. Characterizing gut microbiota dynamics in *Penaeus monodon* fed probiotics using 16S rRNA sequencing. **Microbial Biotechnology** 16(2): 407-419.
- Feng, Y., Liu, Y., Zhang, M. and Wang, J. 2020. Dietary supplementation of probiotics improves immune response and disease resistance in shrimp. **Aquaculture Reports** 17: 100361.
- Gao, X., Zhang, J., Li, Y. and Chen, L. 2021. Dietary probiotics modulate gut microbiota and enhance feed conversion and intestinal integrity in *Litopenaeus vannamei*. **Aquaculture Reports** 21: 100812.
- Hai, N.V. 2015. The use of probiotics in aquaculture. **Journal of Applied Microbiology** 119(4): 917-935.
- Han, J.E., Tang, K.F.J. and Lightner, D.V. 2022. Field and experimental evidence of *Vibrio parahaemolyticus* causing shrimp disease. **Journal of Invertebrate Pathology** 186: 107637.
- Hosein, S.H., Khalili, M., Esteban, M.A. and Ringø, E. 2021. Probiotics as means of diseases control in aquaculture, a review of current knowledge and future perspectives. **Frontiers in Microbiology** 12: 634973.
- Huisakul, M., Chaitanawisuti, N. and Natsukari, Y. 2007. Influence of dietary *Lactobacillus* spp. on gut microbiota in *Penaeus monodon*. **Journal of the World Aquaculture Society** 38(2): 201-209.
- Kesarcodi-Watson, A., Kaspar, H., Lategan, M.J. and Gibson, L. 2012. Probiotics in aquaculture: The need, principles, and mechanisms of action and screening processes. **Aquaculture** 274(1-4): 1-14.
- Khanjani, M.H., Mozanzadeh, M.T. and Gisbert, E. 2024. Probiotics, prebiotics, and synbiotics in shrimp aquaculture: Their effects on growth performance, immune responses, and gut microbiome. **Aquaculture Reports** 38: 102362.
- Kim, Y.J., Kim, S.W. and Lee, S.M. 2022. Modulation of gut microbiota and immune response in shrimp by dietary lactic acid bacteria. **Aquaculture Reports** 25: 101073.
- Kongnum, K. and Hongpattarakere, T. 2012a. Effect of probiotic bacteria isolated from the gastrointestinal tract of wild shrimp on immune response and disease resistance. **Fish & Shellfish Immunology** 32(1): 67-76.
- Kongnum, K. and Hongpattarakere, T. 2012b. Effect of *Lactobacillus plantarum* isolated from digestive tract of wild shrimp on growth and survival of white shrimp (*Litopenaeus vannamei*) challenged with *Vibrio harveyi*. **Fish & Shellfish Immunology** 32(2): 170-177.
- Lai, H.C., Ng, T.H., Ando, M., Lee, C.T., Chen, I.T., Chuang, J.C., Mavvel, D., Chang, S.H., Huang, Y.T. and Wang, H.C. 2015. Pathogenesis of acute hepatopancreatic necrosis disease caused by *Vibrio parahaemolyticus* in shrimp. **Developmental & Comparative Immunology** 52: 77-84.
- Leano, E.M. and Mohan, C.V. 2020. Early mortality syndrome in shrimp: A review. **Asian Fisheries Science** 33(2): 85-96.
- Li, W., Li, X., Chen, Y. and Zhang, Y. 2022. Effects of dietary encapsulated probiotics on growth, intestinal health and immune response in shrimp. **Fish & Shellfish Immunology** 125: 220-228.
- Lulijwa, R., Rupia, E.J. and Alfaro, A.C. 2020. Probiotics and their effects on gut microbiota of aquatic animals. **Applied Microbiology and Biotechnology** 104(17): 7255-7271.
- Martínez-Porchas, M., Vargas-Albores, F. and Miranda-Baeza, A. 2021. Next-generation sequencing in aquaculture: Application and perspectives. **Aquaculture Reports** 19: 100611.
- Nguyen, T.H., Vo, H.T., Pham, T.L. and Doan, H.V. 2019. Effects of dietary lactic acid bacteria on immune response and disease resistance in black tiger shrimp (*Penaeus monodon*). **Aquaculture Research** 50(5): 1218-1229.
- Nguyen, H.T., Trinh, T.L., Nguyen, T.T.H., Nguyen, H.Y.N. and Nguyen, P.T. 2025a. Effect of dietary extracellular polymeric substances on shrimp immune responses. **Veterinary Integrative Sciences** 23(3): e2025061-1-16.
- Nguyen, H.T., Trinh, T.L., Nguyen, T.T.H., Nguyen, H.Y.N. and Nguyen, P.T. 2025b. Probiotic supplementation enhances resistance against *Vibrio* in shrimp. **Aquaculture Reports** 28: 101298.
- Ringø, E., Hoseinifar, S.H., Ghosh, K., Van Doan, H., Beck, B.R. and Song, S.K. 2020. Probiotic use in aquaculture: Importance of gut microbiota modulation. **Reviews in Aquaculture** 12(2): 234-256.
- Sirikharin, R., Thitamadee, S. and Limsuwan, C. 2022. Effects of encapsulated *Bacillus* spp. on gut microbiota and disease resistance in *Penaeus monodon* under semi-intensive culture. **Thai Journal of Aquatic Animal Health** 16(2): 45-56.
- Soundarapandian, P. and Velmurugan, C. 2008. Biochemical composition of the white shrimp *Litopenaeus vannamei* (Boone) collected from pond culture. **American-Eurasian Journal of Scientific Research** 3(5): 191-194.
- Sung, H.H., Lin, Y.C., Chen, C.K. and Chiang, S.R. 2021. Dietary supplementation of lactic acid bacteria improves resistance to *Vibrio* in shrimp. **Aquaculture Reports** 19: 100580.
- Tran, D.T., Nguyen, T.Q., Pham, H.L. and Le, H.V. 2023. Encapsulated probiotics enhance growth and intestinal health in Pacific white shrimp under intensive farming conditions. **Aquaculture** 570: 739418.
- van de Braak, C.B.T., Botterblom, M.H.A., Taverne, N., Rombout, J.H.W.M., Huisman, E.A. and van der Knaap, W.P.W. 2002. The roles of haemocytes and the lymphoid organ in the clearance of injected *Vibrio* bacteria in *Penaeus monodon* shrimp. **Fish & Shellfish Immunology** 13(4): 293-309.

- Vega-Carranza, A.S., Escamilla-Montes, R., Fierro-Coronado, J.A., Diarte-Plata, G., Guo, X., García-Gutiérrez, C. and Luna-González, A. 2024. Investigating the effect of bacilli and lactic acid bacteria on water quality, growth, survival, immune response, and intestinal microbiota of cultured *Litopenaeus vannamei*. **Animals** 14(18): 2676.
- Vieira, F.N., Buglione-Neto, C.C., Ferrarezi, J.V. and Seiffert, W.Q. 2021. Encapsulated probiotics improve intestinal microbiota stability and disease resistance in shrimp. **Fish & Shellfish Immunology** 114: 56-63.
- Xiong, J.B. 2024. Updated roles of the gut microbiota in exploring shrimp etiology and health management. **Microbiology Spectrum** 12(2): e00965-24.
- Zhang, Y., Liu, W., Chen, X. and Wang, Q. 2018. Effects of dietary probiotic supplementation on growth and immune response of *Penaeus monodon*. **Aquaculture Research** 49(10): 3478-3486.
- Zheng, Y., Yu, M., Liu, J., Qiao, Y. and Wu, S. 2017. Effects of dietary probiotics on gut microbiota composition and growth performance in aquaculture species. **Microbial Biotechnology** 10(6): 1345-1356.
- Zhou, Q., Zhang, Q., Wang, Y. and Xia, X. 2023. Probiotic *Lactobacillus* improves immunity and disease resistance in black tiger shrimp. **Aquaculture International** 31(1): 175-188.
- Zokaeifar, H., Balcázar, J.L., Saad, C.R., Kamarudin, M.S., Sijam, K., Arshad, A. and Nejat, N. 2012. Effects of dietary probiotic supplementation on the immune response and disease resistance of *Penaeus monodon*. **Fish & Shellfish Immunology** 32(5): 750-755.