



Effects of Dietary Algae Supplementation on Growth, Hepatopancreatic Histopathology, and Disease Resistance in Post-Larval White Shrimp (*Litopenaeus vannamei*)

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Abstract

Algae have been increasingly incorporated into aquaculture feeds as functional additives with dual roles as immunostimulants and growth enhancement. Various species serve as rich reservoirs of essential nutrients and bioactive constituents—such as vitamins, antioxidants, phycocyanin, minerals, amino acids, carotenoids, proteins, and fatty acids—that collectively promote growth and stimulate immunity. A 28 days feeding trial was conducted to assess the impact of different algae-based supplements on growth performance, hepatopancreatic histology, and resistance to *Vibrio parahaemolyticus* in post-larval *Litopenaeus vannamei*. Shrimp were divided into 4 groups: control (F0) received the basal diet alone, while treatment groups were fed the basal formulation supplemented with 5 g/kg of dried *Ulva* sp. (F1), *Sargassum* sp. (F2), and *Spirulina platensis* (F3). The results revealed that the F3 group (*S. platensis*) achieved the greatest improvement in final body weight, weight gain, average daily gain (ADG), and feed conversion ratio (FCR) compared to F0, F1, and F2 ($p \leq 0.05$). Following a 168-h *V. parahaemolyticus* challenge, shrimp in the F3 group exhibited the lowest cumulative mortality rate (6.60%), significantly lower than that of all other treatments ($p \leq 0.05$). Histological examination of the hepatopancreas revealed superior tissue integrity and a marked reduction in pathogen burden within the F3 group ($p \leq 0.05$). In summary, the study highlights the potential of algae as functional additives in shrimp feed demonstrating their capacity to enhance growth performance, induce beneficial histological alterations, and enhance resistance to *V. parahaemolyticus*. Among the algae tested, *S. platensis* proved to be the most effective supplement.

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Introduction

Pacific white shrimp (*Litopenaeus vannamei*) are regarded as one of Thailand's most economically significant aquatic species. Over the past decade, Thailand has consistently exported frozen Pacific white shrimp and related products to the global market, with an average annual production of approximately 200,000 tons and a peak of 400,000 tons recorded in 2010 (Department of Fisheries, 2024). However, recent years have seen a decline in production, primarily due to disease outbreaks—most notably bacterial infections—that have severely impacted shrimp cultivation in both nursery and grow-out ponds. Among these, *Vibrio parahaemolyticus* is particularly detrimental, as it causes Acute Hepatopancreatic Necrosis Diseases (AHPND) (Poolkhet et al., 2023). Historically, the aquaculture industry has relied on chemotherapeutic agents for disease prevention and treatment in shrimp culture systems (Nolasco-Alzaga et al., 2025). Nevertheless, these conventional methods pose serious ecological and public health risks. The extensive use of chemical compounds contributes to environmental contamination, and may lead to bioaccumulation and residual toxicity in humans. Furthermore, the indiscriminate application of antibiotics has facilitated the emergence of resistant bacterial strains (Ma et al., 2020), thereby diminishing the long-term effectiveness of such treatments.

To address these limitations, recent research has shifted toward the exploration of natural bioactive materials, including medicinal plants, algae, and plant-derived herbal remedies. These substances are increasingly recognized as promising alternatives for disease management in penaeid shrimp aquaculture (dos Luiz et al., 2023). This paradigm shift has led to the adoption of immunostimulatory compounds that enhance innate immune responses and bolster resistance to pathogens in cultured shrimp. Current findings support the strategic use of immunostimulants as an environmentally sustainable approach to improving immunocompetence and disease resilience in intensive aquaculture systems (Nolasco-Alzaga et al., 2025).

Multicellular algae that have emerged as valuable resources in aquaculture due to their rich content of bioactive compounds, such as laminarin, fucoidan, carrageenan, polysaccharide, and alginate, all of which exhibit immunostimulant properties (Luiz et al., 2023). These compounds possess strong antioxidant and antibacterial activities, making them suitable for both

therapeutic and prophylactic applications in aquaculture (Ma et al., 2020). The shrimp immune system has been shown to respond positively to diets incorporating various natural and cultured algae. For instance, brown algae (*Sargassum* spp.) have been identified as a source of polysaccharides with therapeutic and antibiotic properties, offering protection against white spot disease (WSD) in *Penaeus monodon* and *Marsupenaeus japonicus* (Immanuel et al. 2012). Similarly, ethanolic extracts of *Ulva* sp. have been reported to enhance innate immunity and resistance to *Vibrio harveyi* in *L. vannamei* (Abdel-Razek et al., 2024). Supplementation with dried *Spirulina* sp. has also demonstrated significant improvements in growth performance, survival rates, and reproductive outcomes across multiple shrimp species, including Pacific white shrimp (*L. vannamei*) (Kizhakkekarammal et al., 2022), khuruma shrimp, *M. japonicus* (Cuzon et al., 2009), black tiger shrimp, *P. monodon* (Sivakumar et al., 2018).

Despite the documented benefits of algae in aquaculture, comprehensive investigations into the effects of whole-cell algae supplementation—particularly *Ulva* sp., *Sargassum* sp., and *Spirulina platensis*—on the growth performance, hepatopancreatic integrity, and disease resistance in post-larval *L. vannamei* remain limited. Therefore, the present study aims to: (1) evaluate the impact of these algae on shrimp growth metrics, (2) assess histopathological changes in the hepatopancreas following bacterial challenge, and (3) determine the resistance of shrimp to *Vibrio parahaemolyticus* infection when fed algae-supplemented diets.

Materials and methods

1. Preparation of algae-supplemented diets

Ulva sp. and *Sargassum* sp. were collected from Kung Krabea Bay, Chanthaburi Province, Thailand. The algal samples were thoroughly rinsed with distilled water to remove sediment and epiphytic materials, then dried in an incubator at 55°C for 12 h. The dried algae were subsequently ground into fine powder using a mortar and pestle. *S. platensis* was obtained from laboratory cultivation. The biomass was filtered through a fine muslin cloth (20- μ m pore size), washed with distilled water, and dried using a freeze dryer.

Each experimental diet was prepared using a re-pelleting process. Specifically, 5 g of dried algae, 50 mL of fish liver oil, 62.5 g of wheat gluten, and water (25% by weight) were combined/kg of commercial

feed. The mixture was compressed into No. 1 size pellets, air-dried on trays, sealed in plastic bags, and stored at 4°C to maintain nutritional integrity. All diets contained approximately 38–40% protein and 11% lipid.

2. Experimental setting

Post-larval Pacific white shrimp (*L. vannamei*), stage PL15, were sourced from Chonburi Province. Prior to the experiment, shrimp were acclimated for 7 days in a 6000 L tank under laboratory conditions and fed a commercial diet.

2.1 Stage 1: Feeding trial period

Following acclimatization, shrimp with an average initial weight of 0.011 ± 0.004 g were randomly distributed into 12 plastic tanks (60 cm × 40 cm × 40 cm) equipped with aerated water systems ($n = 144/\text{tank}$). Shrimp were assigned to 4 dietary groups with 3 replicates each: F0 (control, basal diet only), F1 (basal diet + 5 g/kg *Ulva* sp.), F2 (basal diet + 5 g/kg *Sargassum* sp.), and F3 (basal diet + 5 g/kg *S. platensis*) (Fig. 1).

Shrimp were fed their respective diets at 10% of body weight/day, divided into 3 equal feedings at 6, 12 and 18 h. The feeding trial lasted 28 days, during which growth performance and survival rates were monitored. At the end of the trial, 5 shrimp from each tank were sampled for histopathological examination of the hepatopancreas (Fig. 1). Throughout the experiment, water quality parameters were maintained as follows: temperature 25–28°C, salinity 15 ppt, pH 7.6–7.8, ammonia and nitrite < 0.05 mg/L, dissolved oxygen \geq

6.0 mg-O₂/L, and alkalinity ~150 mg-CaCO₂/L.

2.2 Stage 2: Challenge period

The challenge test was conducted immediately following the 28-day feeding trial. Shrimps were exposed to a virulent pathogenic strain of *V. parahaemolyticus* (Vp) previously isolated from AHPND-infected *L. vannamei*. The bacterial strain was cultured in Tryptic Soy Broth (TSB) supplemented with 1.5% NaCl for 24 h at 32°C. The bacterial suspension was centrifuged, and the pellet was washed repeatedly with sterile 1.5% NaCl solution. Subsequently, the pellet was resuspended spectrophotometrically to a concentration level of 10¹⁰ CFU/mL, based on optical density of 600 nm.

60 shrimp/experimental group (3 replicates) were maintained in 12 transparent plastic aquaria containing 10 L of seawater 15 ppt salinity. Both treatment and control groups were immersed in the virulent Vp suspension at a final concentration of 10⁶ CFU/mL at 28°C for 6 h. After exposure, the water was replaced several times to prevent bacterial contamination. Mortality was monitored for 5 days (168 h) (Fig. 1) until no further deaths occurred. Dead and surviving shrimp were collected to assess Vp infection and bacterial loads via nested PCR and quantitative real-time PCR, respectively. Cumulative mortality for each group was calculated using the formula:

$$\text{Cumulative mortality rate (\%)} = (\text{number of dead shrimp} / \text{total number of shrimps}) \times 100$$

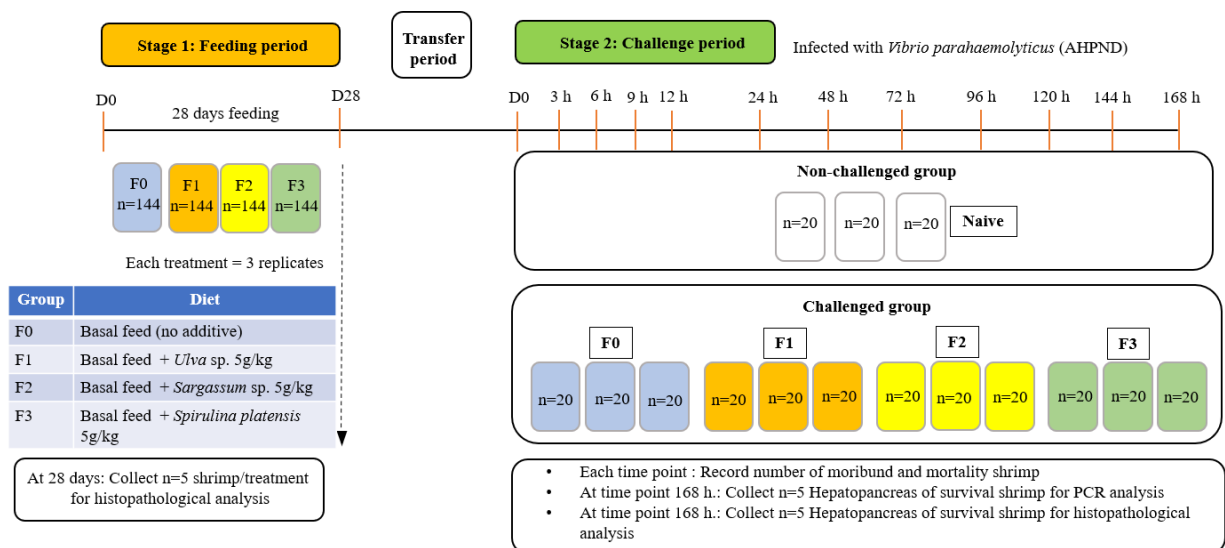


Fig. 1 Experimental design showing feeding and challenge stages to evaluate the effects of algae-supplement diets on Pacific white shrimp (*L. vannamei*). Treatments included control, *Sargassum* sp., *Ulva* sp., *S. platensis*, followed by bacterial challenge with *Vibrio parahaemolyticus*

3. Growth performance and feed efficiency

Shrimp from all experimental groups were sampled weekly to assess growth performance parameters. 10 individuals were randomly collected from each group. The evaluated parameters included body weight gain (BWG), average daily gain (ADG), survival rate (SR), feed intake (FI), and feed conversion ratio (FCR) (Kizhakkemammal et al. 2022).

Body weight gain (BWG; g) = Weight_{Final} (g) - Weight_{Initial} (g)

Average daily gain (ADG; g/shrimp/day) = (M_f - M_i) / D

Where, M_f = final weight (g), M_i = initial weight (g), D = duration of experiment (days)

Survival rate (SR; %) = (N₁ / N₂) × 100

Where, N₁ = number of surviving shrimps, N₂ = initial shrimp stock

Feed intake (FI; g) = Total feed consumed during the 28 days (no residual feed observed in any treatment or control)

Feed conversion ratio (FCR) = FI (g)/BWG (g)

4. Hepatopancreatic histopathological study

At the end of the 28-day feeding trial, 5 shrimp from each experiment were collected. At 168 h post-challenge, 5 surviving shrimp/group were also sampled. Hepatopancreas tissues were fixed in Davidson's solution for 24 h, dehydrated through ascending alcohol concentrations, cleared by xylene, embedded in pure paraplast wax, and sectioned at 5 μm of thickness using a rotatory microtome. Slides were stained with hematoxylin and eosin (H&E) and examined under a compound microscope (Modified from Tran et al., 2013).

5. Determination of *V. parahaemolyticus* infection post-challenged using nested PCR and quantitative real-time PCR

At 168 h post-challenge, surviving shrimp were randomly sampled. DNA was extracted from hepatopancreas tissue using a commercial kit (DNeasy Blood & Tissue Kit, Qiagen). For nested PCR, 100 ng of total DNA/reaction was analyzed following the method of Dangtip et al. (2015). For quantification of *V. Parahaemolyticus* infection levels, DNA template 10 ng of DNA/reaction was assessed using quantitative real-time PCR, based on protocols from the OIE and Han et al. (2015). DNA samples (n = 5) from each tank were pooled and used as templates, with all reactions performed in triplicate.

6. Statistical analysis

All raw data were analyzed using one-way analysis

of variance (ANOVA). Significant differences among treatments were determined using Duncan's multiple range test at p<0.05 (ANOVA, SPSS Version 20, IBM Corp., USA).

Results and discussion

1. Growth performance of shrimp

One hundred percent of survival rates were found on all treatments and control after 28 day of feeding trial. The maximum final body weight (0.205±0.003 g) and weight gain (0.194±0.009 g) of the shrimp was found for the F3 group (Basal diet+S. *platensis*) diet. Also, the growth performance of F3 group was significantly higher than other groups (p≤0.05), as shown by the highest average daily gain (ADG) among the groups. In addition, FCR of the shrimp in F3 group (1.44±0.03) tended to be lower than in the algae treatments and control, and there were significant differences among treatments (p≤0.05). Survival rates of the experimental shrimp were 100% in all treatments including control. From this study, it shows that feed supplemented with *Spirulina* algae have positive effects on shrimp growth as shown in Table 1.

Table 1 Growth performance and feed utilization of *L. vannamei* post-larvae fed diets supplemented with different algae for 28 days

Treatment	F0 (Control: Basal diet only)	F1 (Basal diet + <i>Ulva</i> sp. 5 g/kg.)	F2 (Basal diet + <i>Sargassum</i> sp. 5 g/kg.)	F3 (Basal diet + <i>Spirulina platensis</i> 5 g/kg.)
Final weight (g)	0.169±0.005 ^b	0.134±0.007 ^c	0.183±0.008 ^b	0.205±0.003 ^a
Weight gain (g)	0.158±0.005 ^b	0.123±0.006 ^c	0.172±0.007 ^b	0.194±0.009 ^a
ADG (g/day)	0.006±0.000 ^a	0.004±0.000 ^b	0.006±0.000 ^a	0.007±0.000 ^a
Survival ^{ns} (%)	100	100	100	100
Feed intake ^{ns} (g)	40.32	40.32	40.32	40.32
FCR	1.77±0.05 ^b	2.28±0.07 ^a	1.62±0.07 ^b	1.44±0.03 ^c

Remark: The different superscript letters within rows indicate significant differences among treatments (p≤0.05). ns indicates a non-significant difference.

The current study demonstrated that the dietary supplementation with 5 g/kg of *S. platensis* significantly enhanced weight gain, average daily growth (ADG), and feed conversion ratio (FCR) of *L. vannamei* post-larvae, with statistically significant (p≤0.05) compared to other treatment groups. These findings are consistent with those of Ahmed et al. (2025), who reported that diets supplemented with 4, 6, and 8 g/kg of *S. platensis* significantly improved weight gain, final body weight, specific growth rate, FCR, and survival rate (p≤0.05) in *L. vannamei*. Similarly, Li et al. (2022) found that dried algae used as a feed supplement enhanced growth

performance and physiological resilience under adverse conditions, including stress and disease.

James et al. (2006) and Patnaik et al. (2006) described the beneficial effects of *Spirulina* supplementation on shrimp health and growth through multiple mechanisms. First, *S. platensis* is rich in essential nutrients such as vitamin B12, antioxidants, phycocyanin, minerals, essential amino acids, carotenoids, and proteins that support optimal growth. It also contains polyunsaturated fatty acids (PUFA), particularly n-3 and n-6 forms, which are vital for bio-membrane synthesis. Second, blue-green microalgae improves intestinal microflora by facilitating the breakdown of indigestible feed components and activating enzymes involved in lipid metabolism (Kizhakkekarammal et al., 2022; James et al., 2006). Additionally, *S. platensis* promotes feed efficiency and growth by stimulating populations of beneficial gastrointestinal bacteria (James et al., 2006).

2. Challenge assay with *V. parahaemolyticus*

The cumulative mortality rates during the challenge period are presented in Fig. 2. Mortality was monitored in both the naive non-challenged and the *V. parahaemolyticus* (Vp) challenged groups. No mortality was observed in the naive non-challenged shrimp throughout the study. The highest mortality rate occurred in the F0 group (56.65%), which was significantly greater than those in the other groups ($p \leq 0.05$). Among the shrimp fed diets supplemented with different additives, the F3 group exhibited the lowest cumulative mortality (6.60%), followed by the F2 (13.30%), F1 (16.65%), and F0 (56.65%). All supplemented groups had significantly lower mortality than the control. These results indicate that dietary supplementation with 5 g/kg of dried algae powder enhances resistance to *V. parahaemolyticus* infection in *L. vannamei*.

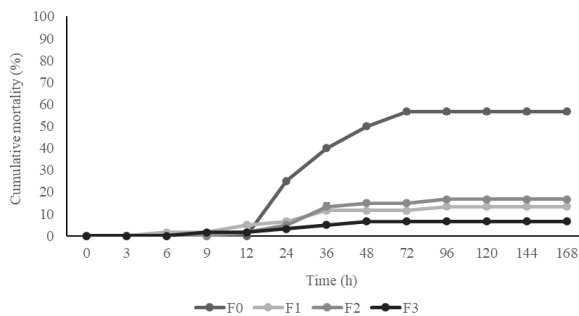


Fig. 2 Cumulative mortality rates of shrimp fed diets supplemented with different types of algae following immersion infection with *V. parahaemolyticus*. Treatments: F0, basal diet (control); F1, basal diet + 5 g/kg *Ulva* sp.; F2, basal diet + 5 g/kg *Sargassum* sp.; F3, basal diet + 5 g/kg *S. platensis*

Algae have long been used as supplementary feed for fish, shrimp, and other animals due to their rich content of protein, C-phycocyanin, carotenoids, minerals, polysaccharides, and vitamins (James et al., 2006). Polysaccharides derived from green and brown algae—such as alginate, carrageenan, fucoidan, laminarin—exhibit various biological activities, including antioxidant, anticancer, anti-inflammatory, antiviral, and antibacterial effects, which enhance shrimp immune responses and provide protection against pathogens (Yudiati et al., 2016).

In the present study, post-challenge survival rates followed the order F3>F2>F1>F0, indicating that dietary supplementation with *S. platensis* conferred the most effective protection against *V. parahaemolyticus* infection, with statistically significant differences ($p \leq 0.05$) compared to other experimental and control groups. This result corroborates earlier research by Ahmed et al. (2025), who observed that shrimp fed diets supplemented by 8 g/kg of *S. platensis* achieved the highest survival rates ($p \leq 0.05$) against *V. parahaemolyticus*. Additionally, Macias-Sancho et al. (2014) reported that replacing fish meal with 25-75% *S. platensis* stimulated immunological parameters in shrimp.

Several factors may explain these outcomes: (i) lyophilized *S. platensis* functions as both prebiotic and antioxidant (Abdelkhalek et al., 2015); (ii) inclusion of *S. platensis* in fish and shellfish diets prevents gut dysbiosis and inhibits pathogen invasion (Celekli et al., 2019); and (iii) *S. platensis* enhance host immune function and acts as immunomodulatory agent (Watanuki et al., 2006; Kizhakkekarammal et al. 2022). Consequently, shrimp fed with algae-supplemented diets exhibit improved resistance against pathogens.

3. Histopathological examination

3.1 Histopathological alteration after the 28-day feeding trial

Histopathological analysis of the shrimp hepatopancreas at the conclusion of the 28-day feeding trial revealed similar hepatopancreatic structures across both control and treatment groups. Histological examination of shrimp fed diets supplemented with dried algae powder showed normal morphology of hepatopancreatic tubules and epithelial cells, with an abundance of F, B and R cells (Fig. 3). However, shrimp fed diets supplemented with *Ulva* sp. exhibited slight epithelial sloughing of tubules and cell necrosis, accompanied by dissolution in some areas of the basement membrane. In contrast, shrimp fed diets

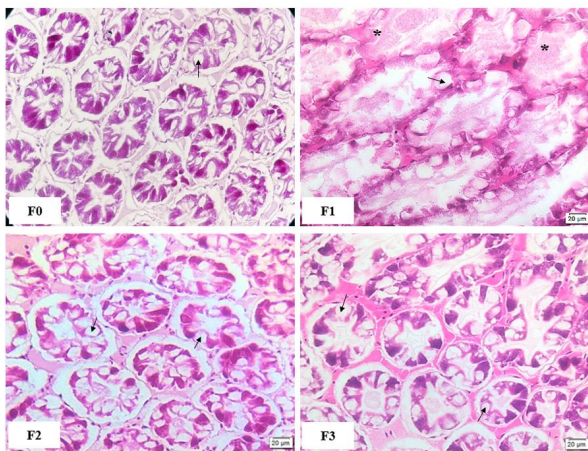


Fig. 3 Histopathological analysis of *L. vannamei* post-larvae hepatopancreas after the 28-d feeding trial. Images show shrimp fed basal diets (F0, control), diets supplemented with *Ulva* sp. (F1), *Sargassum* sp. (F2), and *S. platensis* (F3). Asterisks indicate hepatopancreatic tubule lining, and arrows indicate B cells. Tissue sections were stained with hematoxylin and eosin (H&E), and viewed at 400 × magnification

containing *Sargassum* sp. and *S. platensis* displayed hepatopancreatic structures comparable to the control group as illustrated in Fig. 3. These findings indicate that dietary supplementation with either *Sargassum* sp. or *S. platensis* did not induce any histopathological alterations in the hepatopancreas of shrimp.

3.2 Histopathological alterations after challenge with *V. parahaemolyticus* (Vp)

This study investigated hepatopancreatic pathology in shrimp fed diets containing various algae species following immersion infection with Vp. After 168 h, hepatopancreatic tissue of surviving shrimp in the control group (F0) exhibited the most severe epithelial sloughing of the tubules. Shrimp fed with the diet supplemented with *Ulva* sp. (F1) showed a reduction in R cells, pronounced under nuclear pyknosis (indicated by triangles), and loss of lumen structures (indicated by arrows). In shrimp fed with *Sargassum* sp. (F2), there was a separation between the myoepithelial layer and the hepatopancreatic epithelium (marked by double arrowheads). The hepatopancreas of shrimp fed diets supplemented with *S. platensis* (F3) appeared normal. These observations indicate that shrimp in the F3 group exhibiting superior hepatopancreatic health and greater resistance to Vp infection compared to other groups, as illustrated in Fig. 4.

The hepatopancreas is the primary organ responsible for the digestive and immune functions in shrimp. It is also the main organ susceptible to damage

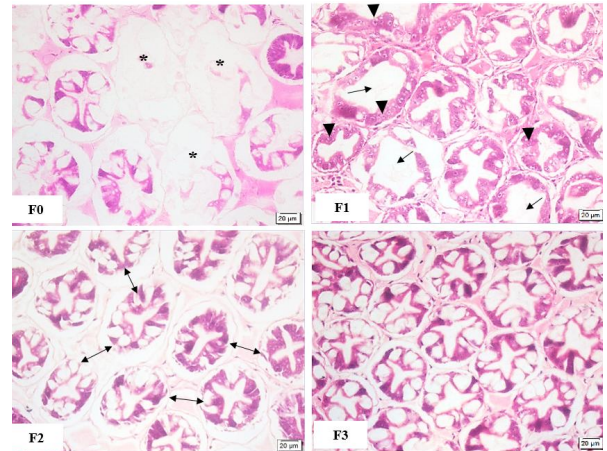


Fig. 4 Histopathological analysis of *L. vannamei* post-larvae hepatopancreas following infection with *V. parahaemolyticus* (Vp). Images show shrimp fed basal diets (F0, control), diets supplemented with *Ulva* sp. (F1), *Sargassum* sp. (F2), and *S. platensis* (F3). Stars indicate hepatopancreatic tubule epithelial sloughing, triangles indicate excessive nuclear pyknosis, arrows indicate disappearance of lumen structures, and double arrowheads indicate separation between myoepithelial layer and epithelium. Tissue sections were stained with hematoxylin and eosin (H&E) and viewed at 400 × magnification

from dietary factors or toxins. When shrimp are infected with virulent pathogenic bacteria, particularly in *V. parahaemolyticus* strains causing Acute Hepatopancreatic Necrosis Diseases (AHPND) or *V. harveyi*, the hepatopancreas is severely affected, leading to necrosis, epithelial cell sloughing, tissue degeneration, and inflammation. These pathological changes result in impaired or complete loss of hepatopancreatic function, ultimately compromising shrimp health and growth. The present study suggests that *S. platensis* is effective in preventing this disease and preserving hepatopancreatic tissue integrity. These findings align with those of Ahmed et al. (2025) who reported that supplementation of shrimp diets with 6 and 8 g/kg of dried *S. platensis* promoted recovery of hepatopancreatic tissue and enhanced resistance to *V. parahaemolyticus* in *L. vannamei*. Additionally, Li et al. (2019) found that *Spirulina* can eliminate harmful chemicals in feed and stimulate the immune system, thereby protecting hepatopancreatic cells from damage. Furthermore, Kizhakkekarammal et al. (2022) attributed the beneficial effects of *S. platensis* to its high content of C-phycoerythrin, which appears to preserve hepatopancreatic tissue and enhance immune function. Consistent with the present study, Setyawan et al. (2021) observed that *L. vannamei* fed sodium alginate derived from *Sargassum* exhibited slight hepatopancreatic damage, including vacuolation and necrosis.

4. Study of infection and bacterial Vp load in shrimp post-challenge

No *V. parahaemolyticus* (Vp) infection was detected in shrimp from the non-challenged (naive) group. In contrast, surviving shrimp fed diets supplemented with all 3 types of algae, as well as those in the control group, tested positive for Vp infection. Quantitative analysis of the Vp copy number/ng of DNA in surviving shrimp at 168 h post-infection revealed the highest bacterial load in the control group (F0) with $8.54 \pm 3.37 \times 10^6$ copies/ng DNA. This was followed by the F2 group (*Sargassum* sp.) with $4.58 \pm 3.67 \times 10^6$ copies/ng DNA, the F1 group (*Ulva* sp.) with $0.83 \pm 0.30 \times 10^6$ copies/ng DNA, and the lowest load observed in the F3 group (*S. platensis*) with $0.22 \pm 0.038 \times 10^6$ copies/ng DNA. Shrimp fed diets supplemented with *Ulva* sp. and *S. platensis* exhibited significantly lower Vp loads compared to the control group ($p \leq 0.05$) (Table 2).

Table 2 Summary of *Vibrio parahaemolyticus* (Vp) infection and copy numbers among challenged groups by nested PCR And quantitative real-time PCR

Treatment	Infection Results by Nested PCR	Load Of Vp by Quantitative Real-Time PCR (Mean Vp copies / 50 ng of DNA \pm SE)
F0 (Control)	+	$8.54 \pm 3.37 \times 10^{6a}$
F1 (Basal diet + <i>Ulva</i> sp. 5 g/kg)	+	$0.83 \pm 0.30 \times 10^{6b}$
F2 (Basal diet + <i>Sargassum</i> sp. 5 g/kg)	+	$4.58 \pm 3.67 \times 10^{6a}$
F3 (Basal diet + <i>S. platensis</i> 5 g/kg)	+	$0.22 \pm 0.038 \times 10^{6c}$

Remark: + = positive for Vp infection. Different superscripts (a, b, c) indicate statistically significant difference among groups within each measurement at the same time point ($p \leq 0.05$).

Shrimp fed diets supplemented with *Ulva* sp. and *S. platensis* showed significantly reduced bacterial loads compared to the control group, indicating that both algal species possess effective antimicrobial properties against pathogenic bacteria. This finding supports the report of Thanigaivel et al. (2016) who described algae as having strong antioxidant capacity and acting as natural medicines. *Ulva* sp. contains sulfated polysaccharides with antimicrobial activity, along with phenolic compounds that exhibit both antioxidant and antimicrobial effects by lysing bacterial cell membranes and inhibiting bacterial adhesion in the host. Additionally, this alga stimulates immunity by activating phenoloxidase enzymes and increasing hemocyte counts, which help the host eliminate pathogens. Kanjana et al. (2011) confirmed the inhibitory effects of *Ulva* sp. extracts against *V. harveyi* in black tiger shrimp. Similarly, Klongklaew et al. (2021) reported that inclusion of *Ulva*

intestinalis extract in Pacific white shrimp diets improved immunity and reduced mortality caused by infection with *V. parahaemolyticus* and viruses. Hejna et al. (2024) found that *Ulva lactuca* extracts demonstrated promising antibacterial and antioxidant effects against pathogenic bacteria.

Notably, this study demonstrated that dietary supplementation with *S. platensis* resulted in the most significant reduction in bacterial load. *Spirulina* contains phycocyanin, a pigment with potent antioxidant and anti-inflammatory properties, as well as gamma-linolenic acid (GLA), a fatty acid that strengthens cell membranes and has anti-inflammatory effects. It also includes antimicrobial compounds that directly inhibit bacterial growth and promote beneficial intestinal bacteria while reducing pathogenic bacteria in shrimp. Ahmed et al. (2025) found that white shrimp fed with 6 and 8 g/kg *S. platensis* supplements exhibited higher survival rates when infected with *V. parahaemolyticus*. Furthermore, Immanuel et al. (2012) reported a considerable reduction of WSSV DNA copy numbers in shrimp fed algae containing fucoidan, supporting the antiviral benefits of algal compounds.

Conclusion

Supplementation of *S. platensis* at 5 g/kg in shrimp diets significantly enhances growth performance, reduces hepatopancreatic tissue damage, and improves resistance to *Vibrio parahaemolyticus* infection in post-larval *L. vannamei*. These findings support the use of *S. platensis* as a functional feed additive to promote sustainable shrimp aquaculture. However, further studies are warranted to optimize dosage, evaluate long-term effects and elucidate the underlying mechanisms of immune enhancement.

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