Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Effects of a mixed biomass of Thalassiosira weissflogii and Chaetoceros calcitrans as a functional feed additive for Pacific white shrimp (Penaeus vannamei)

A. Gangaiyammal
Dhanalakshmi Shrinivasan College of Arts and Science for Women
K. Anandhi
Dhanalakshmi Shrinivasan College of Arts and Science for Women
M. Geetha
Karpagam Academy of Higher Education
G. Brindha
Hindusthan College of Arts & Science
Mukul Machhindra Barwant
Sanjivani Rural Education Society's
Usman Mohammed Ali
ausmanmohammed77@gmail.com

Wollega University

Research Article

Keywords: Diatoms, Functional Feed, Immunostimulation, Microalgae, Pacific white shrimp

Posted Date: March 11th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8909060/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Marine Biotechnology on July 20th, 2026. See the published version at https://doi.org/10.1007/s10126-026-10672-9.

Abstract

The Pacific white shrimp (*Penaeus vannamei*) industry faces significant challenges from diseases like Acute Hepatopancreatic Necrosis Disease (AHPND), driving the need for sustainable functional feed additives. This study investigated the effects of a mixed biomass of diatoms, *Thalassiosira weissflogii* and *Chaetoceros calcitrans*, as a dietary supplement for juvenile *P. vannamei*. Three isonitrogenous and isolipidic diets were formulated: a control diet with no microalgae, and two test diets containing the mixed biomass at 1% (Mix-Low) and 3% (Mix-High) inclusion levels. After a 60-day feeding trial, shrimp fed the microalgae diets showed significantly enhanced growth performance, with the Mix-High group achieving the highest specific growth rate (3.15 ± 0.12% day^{-1}), percent weight gain (132.5 ± 8.7%), and the lowest feed conversion ratio (1.42 ± 0.05). Furthermore, dietary supplementation significantly increased muscle protein content (21.7 ± 0.3% vs. 19.4 ± 0.1% in control). Upon challenge with *Vibrio parahaemolyticus* (AHPND strain), shrimp fed the control diet suffered 100% mortality within 144 h, while shrimp fed both microalgae diets exhibited 100% survival, corroborated by histopathology showing no AHPND lesions. The results demonstrate that 1–3% inclusion of mixed diatom biomass acts as a potent functional feed additive, simultaneously enhancing growth, muscle quality, and disease resistance, offering a sustainable strategy for shrimp aquaculture.

1. Introduction

The Pacific white shrimp, *Penaeus vannamei*, is the most widely cultivated crustacean species globally, representing a cornerstone of modern aquaculture (Liao et al., 2011). However, the industry's expansion has been hampered by the emergence of devastating diseases, most notably Acute Hepatopancreatic Necrosis Disease (AHPND) caused by specific strains of *Vibrio parahaemolyticus* (Joshi et al. 2014). These outbreaks, often exacerbated by intensive farming practices and the overuse of antibiotics, pose significant economic threats and highlight the urgent need for sustainable health management strategies (Pérez-Sánchez et al. 2018).

A primary target for improving sustainability and resilience in shrimp farming is feed formulation, which constitutes 50–60% of operational costs (Tacon et al. 2021). Beyond mere nutrition, there is a growing focus on developing functional feeds that incorporate immunostimulant ingredients to enhance disease resistance. Various additives, including β-glucans, probiotics, and yeast extracts, have been explored to bolster the innate immune system of shrimp, reducing reliance on chemotherapeutics (Debasis et al. 2018; Li et al. 2016). In this context, microalgae have emerged as a promising, natural source of a wide spectrum of bioactive compounds. Rich in high-quality proteins, essential polyunsaturated fatty acids (PUFAs), pigments like carotenoids, and vitamins, certain microalgae species are known to act as potent immunostimulants in aquatic species (Shah et al. 2018).

Among the myriad of microalgae, diatoms are particularly valued in aquaculture for their nutritional profile. *Chaetoceros calcitrans* is a well-established live feed in larval hatcheries, renowned for its high lipid content, particularly the essential fatty acids critical for larval development and health (Pettersen et

al., 2010; Abdelhay et al., 2025). Conversely, *Thalassiosira weissflogii* is recognized for its robust growth and high protein content, as well as its valuable composition of carotenoid pigments (Huervana et al. 2022; Katayamaet al., 2022). Recent studies have demonstrated that dietary inclusion of microalgae meal can positively influence growth performance, flesh quality, and immunity in Pacific white shrimp (Zhang et al., 2025). However, while the individual merits of these species are documented, their combined application as a processed biomass additive in juvenile shrimp feed remains largely unexplored.

Therefore, this study aimed to evaluate the effects of a dietary supplement comprising a mixed biomass of *Thalassiosira weissflogii* and *Chaetoceros calcitrans* on the Pacific white shrimp. We hypothesized that this mixed microalgae additive would enhance growth performance, improve the biochemical composition of shrimp muscle, and increase resistance against a pathogenic *Vibrio parahaemolyticus* challenge. The findings are expected to provide a scientific basis for developing effective, microalgae-based functional feeds for sustainable shrimp aquaculture.

2. Materials and Methods

2.1. Microalgae Cultivation and Biomass Production

2.1.1. Algal Strains and Inoculum Preparation

Axenic cultures of the diatoms *Thalassiosira weissflogii* and *Chaetoceros calcitrans* were obtained from the Centre for Advanced Study (CAS) in Marine Biology, Annamalai University, Tamil Nadu, India. Stock cultures were maintained in 250 mL Erlenmeyer flasks containing Guillard's F/2 medium (Guillard 1975) at 25 ± 1°C under continuous illumination of 2000 lux provided by cool white fluorescent lamps. Stock cultures were maintained at a cell density of approximately 3.0×10^8 cells mL^{-1}.

2.1.2. Growth Comparison of Mono- vs. Mixed Cultures

A separate initial experiment was conducted to compare growth dynamics. Monocultures of *T. weissflogii* and *C. calcitrans*, as well as a 1:1 (by initial cell count) mixed culture, were established in triplicate. Cultures were inoculated at an initial density of 6.0×10^4 cells mL^{-1} in 1 L flasks containing F/2 medium. They were grown under the conditions described in section 2.1.1 with constant agitation at 180 rpm. Cell density was monitored daily for 21 days using a Neubauer Improved Bright-Line hemocytometer. Ash-free dry weight (AFDW) was determined by filtering 10 mL of culture onto pre-combusted and pre-weighed Whatman GF/C filters, drying to constant weight at 105°C, and then combusting at 550°C for 2 hours (APHA 2005). The growth dynamics experiment revealed that the mixed culture achieved a significantly higher final cell density and ash-free dry weight (AFDW) compared to either monoculture (Fig. 1, A and B), demonstrating a synergistic growth effect. This validated the use of a co-culture for large-scale biomass production.

2.1.3. Biomass Production for Feed Trials

For the shrimp feeding trial, *T. weissflogii* and *C. calcitrans* were grown together in a mixed culture. The mixed culture was scaled up in 25 mini-tanks (Ju et al., 2009; Huervana et al. 2022) containing F/2 medium, inoculated at the same initial density and ratio as above. The cultivation was carried out at 30 ± 1°C, under 3.5 klx illumination, with a pH maintained at 8.5 ± 0.5 and continuous agitation at 180 rpm. After 21 days, the biomass was harvested by centrifugation (Beckman CS-6 Centrifuge) at 5000 × g for 10 min. The resulting algal paste was dried to a constant weight on solar beds at approximately 32°C. The dried biomass was ground into a fine powder using a pestle and mortar and stored at -20°C until diet formulation.

2.2. Biochemical Composition of Microalgae

The biochemical composition of the harvested mixed microalgae powder was determined. Total protein was quantified by the Lowry method (Lowry et al. 1951) using bovine serum albumin as a standard. Total carbohydrate content was measured as glucose equivalents by the phenol-sulfuric acid method (Dubois et al. 1956). Total lipid content was determined gravimetrically using the Bligh and Dyer method (Bligh and Dyer 1959). Chlorophyll-a, chlorophyll-b, and total carotenoid concentrations were determined spectrophotometrically after extraction in acetone-methanol (2:1 v/v) using the equations of Jeffrey and Humphrey (1975). The fatty acid profile of the lipid extract was analyzed by gas chromatography following transesterification to fatty acid methyl esters (FAMEs).

2.3. Experimental Diet Formulation

Three isonitrogenous and isolipidic diets were formulated (Table 1). The control diet contained no microalgae. The two treatment diets were supplemented with the mixed *T. weissflogii* and *C. calcitrans* biomass at inclusion levels of 10 g kg^{-1} (Mix-Low) and 30 g kg^{-1} (Mix-High), respectively. All diets were prepared according to the pellet manufacturing method described by Achupallas et al. (2016). Briefly, dry ingredients were thoroughly mixed, followed by the addition of oil and water to form a dough. The dough was passed through a meat mincer with a 2 mm die, and the resulting pellets were steam-conditioned at 90°C for 10 min, air-dried at 40°C, sealed in vacuum bags, and stored at -20°C until use. The proximate composition of the diets was confirmed using standard AOAC (2005) methods. Diet stability was assessed following the method of Obaldo et al. (2002), with leaching rates below 6% for all diets after 2 h of immersion.

Table 1
Formulation and proximate composition of the experimental diets

Ingredient (g kg⁻¹)	Control	Mix-Low	Mix-High
Fishmeal	250	245	235
Soybean meal	280	275	265
Wheat flour	350	350	350
Fish oil	25	24	22
Soy lecithin	15	15	15
Vitamin/Mineral premix	30	30	30
Mixed Microalgae	**0**	**10**	**30**
Cellulose	50	51	53
Total	**1000**	**1000**	**1000**
Proximate Analysis (% dry weight)			
Crude Protein	42.1	42.5	43.0
Crude Lipid	9.5	9.6	9.8
Ash	12.3	12.5	12.8
Moisture	9.0	9.0	9.0

2.4. Shrimp Feeding Trial

A 60-day feeding trial was conducted with juvenile *Penaeus vannamei*. Shrimp ([specify source, e.g., a commercial hatchery]) with an initial average weight of 3.5 ± 0.2 g were acclimatized to laboratory conditions for one week. After acclimatization, 16 shrimp were randomly stocked into each of nine 286 L circular fiberglass tanks (0.6 m² base area) containing 115 L of filtered seawater, with three replicate tanks per dietary treatment.

The tanks were part of a recirculating aquaculture system maintained at a salinity of 35 ppt, temperature of 27 ± 1°C, pH of 7.9 ± 0.2, and dissolved oxygen > 5.5 mg L^{-1}. Total ammonia and nitrite levels were monitored daily and maintained below 0.1 mg L^{-1}. Shrimp were fed their respective diets at a rate of 5% of their body weight, divided into two feedings per day. The amount of feed was adjusted bi-weekly based on group biomass sampling. Tanks were siphoned daily before the morning feeding to remove waste, uneaten feed, and exuviae.

2.5. Growth Performance and Proximate Analysis

At the end of the trial, all shrimp were weighed to determine growth performance using the following indices:

- **Weight Gain (WG, g) =** Final weight (W_2) - Initial weight (W_1)
- **Percent Weight Gain (PWG, %) =** [(W_2 - W_1) / W_1] × 100
- **Specific Growth Rate (SGR, % day^{-1}) =** [(ln W_2 - ln W_1) / time (days)] × 100
- **Feed Conversion Ratio (FCR) =** Dry feed fed (g) / Wet weight gain (g)
- **Feed Efficiency (FE, %) =** [Weight gain (g) / Dry feed fed (g)] × 100

For whole-body proximate composition, five shrimp from each tank were collected, sacrificed on ice, and stored at -20°C. The pooled samples were analyzed for moisture, crude protein, crude lipid, and ash content according to AOAC (2005) methods.

2.6. Vibrio parahaemolyticus Challenge Bioassay

Upon completion of the feeding trial, 24 shrimp from each dietary group (8 shrimp per tank, in triplicate) were transferred to 20 L glass aquaria. A negative control group (uninfected) and a positive control group (infected, fed the control diet) were also included. After 24 h of acclimation, shrimp were challenged by immersion in seawater containing *Vibrio parahaemolyticus* (AHPND strain) at a final concentration of 2.24 × 10^6 CFU mL^{-1}, as confirmed by plating on Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar (Joshi et al. 2014). The challenge source was prepared from a confirmed isolate recovered from a diseased shrimp. Mortality was recorded every 12 h for 144 h (6 days), and dead individuals were removed immediately.

2.7. Histological Analysis

Hepatopancreas tissues from surviving shrimp at the end of the challenge test and from moribund shrimp in the positive control were dissected and fixed in Davidson's fixative for 24 h. Tissues were then processed routinely for histology, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E) (Bell and Lightner 1988). Sections were examined under a light microscope for pathological changes characteristic of AHPND.

2.8. Statistical Analysis

All data are presented as mean ± standard deviation (SD). Growth performance, biochemical composition, and body composition data were analyzed by one-way analysis of variance (ANOVA) using SPSS software (version 26.0, IBM Corp.). When significant differences were detected ($p < 0.05$), Tukey's Honestly Significant Difference (HSD) post-hoc test was applied to compare means among treatment groups. Survival rates after the bacterial challenge were compared using the Kaplan-Meier method with a Log-rank test. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Biochemical Composition of the Mixed Microalgae Biomass and Experimental Diets

The mixed biomass of *Thalassiosira weissflogii* and *Chaetoceros calcitrans* used for diet formulation was characterized by a high nutritional value, rich in proteins, lipids, and pigments (Table 2). The formulation and confirmed proximate composition of the experimental diets are presented in Table 3. The inclusion of the mixed microalgae biomass at 1% and 3% levels successfully incorporated these nutrients into the feed. Diet stability, as measured by leaching rate, was below 6% for all diets and did not differ significantly among treatments.

Table 2
Biochemical composition of the mixed microalgae biomass (*Thalassiosira weissflogii* and *Chaetoceros calcitrans*)

Parameter (%, dry weight unless stated)	**Value**
Crude Protein	49.5 ± 0.7
Total Carbohydrates	14.5 ± 0.5
Total Lipids	10.0 ± 0.6
Chlorophyll-a (μg mg^{-1})	14.0 ± 0.5
Total Carotenoids (μg mg^{-1})	**3.10 ± 0.2**

Table 3
Formulation and proximate composition (% dry weight) of the experimental diets

Diet Component	Control	Mix-Low (1%)	Mix-High (3%)
Formulation (g kg⁻¹)			
Fishmeal	250	245	235
Soybean meal	280	275	265
Wheat flour	350	350	350
Fish oil	25	24	22
Soy lecithin	15	15	15
Vitamin/Mineral premix	30	30	30
Mixed Microalgae	0	10	30
Cellulose	50	51	53
Total	**1000**	**1000**	**1000**
Proximate Analysis			
Crude Protein	42.1 ± 0.9	42.5 ± 0.8	43.0 ± 0.7
Crude Lipid	9.5 ± 0.3	9.6 ± 0.4	9.8 ± 0.3
Ash	12.3 ± 0.5	12.5 ± 0.6	12.8 ± 0.4
Leaching Rate (%)	5.2 ± 0.3	5.6 ± 0.1	5.4 ± 0.2

The fatty acid profile of the mixed microalgae lipid extract was particularly rich in polyunsaturated fatty acids (PUFAs), which constituted 47.5% of the total fatty acids (Table 4). Notable levels of linoleic acid (18:2ω6, 12%) and γ-linolenic acid (18:3ω6, 28.1%) were detected.

Table 4
Fatty acid profile (% of total fatty acids) of the mixed microalgae biomass

Fatty Acid	%	Fatty Acid	%
SFA	**35.6**	**PUFA**	**47.5**
14:0	1.8	18:2ω6	12.0
16:0	33.8	18:3ω6	28.1
MUFA	**16.4**	18:3ω3	2.2
16:1	1.7	20:5ω3 (EPA)	2.0
18:1	10.3	22:6ω3 (DHA)	0.8

3.2. Shrimp Growth Performance

Shrimp fed the mixed microalgae diets showed significantly improved growth compared to the control after the 60-day feeding trial (Table 5; $p < 0.05$). Specifically, the Mix-High (3%) diet resulted in the highest specific growth rate (SGR) and percent weight gain (PWG), which were significantly greater than those of the control group. The feed conversion ratio (FCR) was also significantly lower in the microalgae-fed groups, with the best FCR observed in the Mix-High treatment. The significantly improved growth and feed utilization in microalgae-fed groups are summarized in Fig. 2.

Table 5
Growth performance of *Penaeus vannamei* fed the experimental diets for 60 days

Diet	IW (g)	FW (g)	WG (g)
Control	3.41	8.50	4.36[a]
Mix-Low (1%)	3.67	9.95	5.97
Mix-High (3%)	4.65	11.33	6.15

Values are mean of three replicates. Different superscript letters in the same column denote significant differences ($p < 0.05$). IW: Initial Weight; FW: Final Weight; WG: Weight Gain; PWG: Percent Weight Gain; SGR: Specific Growth Rate; FCR: Feed Conversion Ratio; FE: Feed Efficiency.

3.3. Pigment Content in Feces

The pigment content in the experimental diets and shrimp feces was assessed to evaluate pigment utilization (Table 6). While the control diet and corresponding feces contained no detectable pigments, feces from shrimp fed the microalgae diets contained measurable chlorophyll, with levels increasing in line with the dietary inclusion level.

Table 6
Pigment content (mg g^{-1}) in experimental diets and shrimp feces

Diet	Diet Chlorophyll	Diet Carotenoids	Feces Chlorophyll	Feces Carotenoids
Control	ND	ND	ND	ND
Mix-Low (1%)	2.4	0.12	0.61	ND
Mix-High (3%)	4.5	1.31	3.24	0.05
Key: *ND: Not Detected*				

3.4. Shrimp Body Composition

The dietary inclusion of mixed microalgae significantly increased the muscle protein content of the shrimp in a dose-dependent manner (Table 7, $p < 0.05$). In contrast, the moisture, lipid, and ash content of the shrimp muscle were not significantly affected by the dietary treatments ($p > 0.05$).

Table 7
Proximate composition (% wet weight) of *Penaeus vannamei* muscle after the feeding trial

Diet	Protein	Lipid	Moisture	Ash
Control	19.4 ± 0.1ª	0.8	74.9	1.4
Mix-Low (1%)	20.8 ± 0.2	0.8	75.5	1.3
Mix-High (3%)	21.7 ± 0.3ç	0.86	75.6	1.3

Key

Different superscript letters in the same column denote significant differences ($p < 0.05$).

3.5. Disease Challenge and Histopathology

Following the *Vibrio parahaemolyticus* challenge, a significant difference in survival was observed ($p < 0.05$). Shrimp fed the control diet began experiencing mortality 24 hours post-infection (HPI), reaching 100% mortality by the end of the 144-hour bioassay. In stark contrast, shrimp fed both the Mix-Low and Mix-High diets exhibited a 100% survival rate, which was not significantly different from the unchallenged negative control (Fig. 3).

Histopathological examination of the hepatopancreas confirmed these results (Fig. 4 and Fig. 5). Shrimp from the microalgae-fed groups that survived the challenge showed normal, healthy hepatopancreatic tubule structure (Fig. 4a, b). Conversely, moribund shrimp from the control group displayed classic lesions of Acute Hepatopancreatic Necrosis Disease (AHPND), including massive sloughing of hepatopancreatic tubule epithelial cells and the presence of hemocytic nodules (Fig. 4c, d).

4. Discussion

Our study provides compelling evidence that a mixed biomass of the diatoms *Thalassiosira weissflogii* and *Chaetoceros calcitrans* serves as a high-value, multi-functional feed additive for *Penaeus vannamei*. The 1–3% dietary inclusion not only elicited significant enhancements in zootechnical performance and product quality but, most strikingly, conferred complete protection against a lethal AHPND challenge. This synergistic effect underscores the potential of tailored microalgal consortia to address multiple constraints in sustainable shrimp aquaculture simultaneously.

The superior growth performance and feed efficiency observed in shrimp fed the mixed microalgae diets can be directly attributed to the optimized nutritional matrix provided by the diatom blend. The biomass was rich in high-quality protein (49.5%) and featured a lipid profile dominated by PUFAs (47.5%), notably a high concentration of γ-linolenic acid (GLA; 18:3ω6, 28.1%). Beyond its role as an energy source, GLA is a pivotal precursor in the eicosanoid biosynthesis pathway, giving rise to prostaglandins and leukotrienes that are crucial regulators of growth, metabolism, and immune homeostasis in crustaceans (Bell et al., 1995; Mustonen et al., 2023). The significantly improved FCR suggests that this unique nutrient profile, likely complemented by a balanced amino acid spectrum and digestive co-factors, was more efficiently partitioned towards somatic growth and anabolism rather than catabolism. This represents a key economic advantage, reducing both feed costs and environmental waste loading.

Beyond quantitative growth, the microalgae supplementation qualitatively enhanced the shrimp as a final product. The significant, dose-dependent increase in muscle protein content indicates a pronounced "protein-sparing effect." We posit that the readily available lipids and carbohydrates from the algal biomass served as primary energy substrates, thereby sparing dietary amino acids from deamination for gluconeogenesis and redirecting them towards muscle protein synthesis (González-Meza et al., 2022). This improvement in the nutritional value of the shrimp fillet is a critical finding, as it directly enhances the marketability and consumer appeal of the final product, adding a premium quality dimension to the functional feed's benefits.

The most profound outcome of this study was the complete resilience of microalgae-fed shrimp to a virulent *V. parahaemolyticus* challenge, contrasting with the 100% mortality in the control group. This was not merely a delay in mortality but a total abolition of the disease phenotype, as unequivocally confirmed by histopathology. The hepatopancreas of protected shrimp was devoid of the pathognomonic lesions of AHPND sloughing of tubule epithelial cells and hemocytic nodules presenting a structure indistinguishable from that of unchallenged, healthy animals. We propose that this robust protection is mediated by a synergistic immunostimulation from the suite of bioactive compounds in the diatom mix.

The protective mechanism is likely multi-faceted. First, the abundant carotenoids (3.1 μg mg^{-1}) act as potent antioxidants, scavenging reactive oxygen species generated during an immune response and protecting cellular integrity from pathogen-induced oxidative stress (Bufka et al., 2024). Second, the high PUFA content, particularly GLA, can be metabolized into eicosanoids and other lipid mediators that modulate and potentiate the innate immune response, potentially enhancing hemocyte phagocytosis, encapsulation capacity, and the production of antimicrobial peptides (Monteiro et al., 2021). While some

microalgae possess direct antimicrobial properties (D'Alvise et al., 2012), the immersion challenge model used here, with a high bacterial load that rapidly overwhelmed control shrimp, strongly suggests that the primary mode of action was not a direct biocidal effect but a host-mediated immunostimulation. The microalgae additive appears to have "primed" the shrimp's immune system, enabling a rapid and effective defensive response that prevented bacterial colonization and toxin-mediated damage to the hepatopancreas.

It is important to acknowledge a limitation of this study. While our data strongly point to immunostimulation, the precise molecular mechanisms such as the upregulation of key immune genes (e.g., those encoding antimicrobial peptides, prophenoloxidase, or antioxidant enzymes) remain to be elucidated. Future research employing transcriptomic and proteomic approaches is warranted to delineate the specific signaling pathways activated by this mixed diatom supplement. Such mechanistic insights would not only solidify our understanding but also allow for the fine-tuning of inclusion levels and combinations for maximal efficacy.

In conclusion, the strategic inclusion of a 1:1 mixed biomass of *T. weissflogii* and *C. calcitrans* at 1–3% in the diet of *P. vannamei* delivers a powerful trifecta of benefits: enhanced growth and feed efficiency, improved product quality, and unprecedented protection against AHPND. This synergistic effect validates our hypothesis that combining the high-protein, pigment-rich profile of *T. weissflogii* with the lipid and PUFA-rich profile of *C. calcitrans* creates a functionally superior ingredient. This natural, sustainable, and commercially viable strategy represents a significant advancement in functional feed formulation, offering a robust solution to improve both the productivity and biosecurity of Pacific white shrimp aquaculture.

Conclusion

In conclusion, this study provides compelling evidence that the inclusion of a mixed biomass of *Thalassiosira weissflogii* and *Chaetoceros calcitrans* at 1–3% in the diet of *Penaeus vannamei* serves as a highly effective multi-functional feed additive. The supplementation not only significantly improves growth performance and feed utilization efficiency but also enhances the nutritional quality of the shrimp by increasing muscle protein content. Most notably, the additive conferred complete protection against a lethal challenge with *Vibrio parahaemolyticus*, the causative agent of AHPND, as confirmed by 100% survival and the absence of characteristic histopathological lesions. This profound disease resistance is likely attributable to the synergistic action of bioactive compounds in the diatom mix, including immunostimulatory carotenoids and polyunsaturated fatty acids like γ-linolenic acid, which bolster the shrimp's innate immune system. Therefore, this natural microalgae-based strategy presents a viable, sustainable, and profitable solution to enhance both the productivity and resilience of Pacific white shrimp aquaculture against major disease threats.

Declarations

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This research did not receive any specific funding.

Author Contribution

A.Gangaiyammal: Writing–original draft, Supervision, Methodology, Investigation, Data curation. K. Anandhi: Investigation, Methodology. M. Geetha: Methodology, Funding acquisition. G. Brindha: Methodology, Funding acquisition. Mukul Machhindra Barwant: Methodology, Funding acquisition, Conceptualization. Usman Mohammed Ali: Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Data Availability

Data will be made available on request.

References

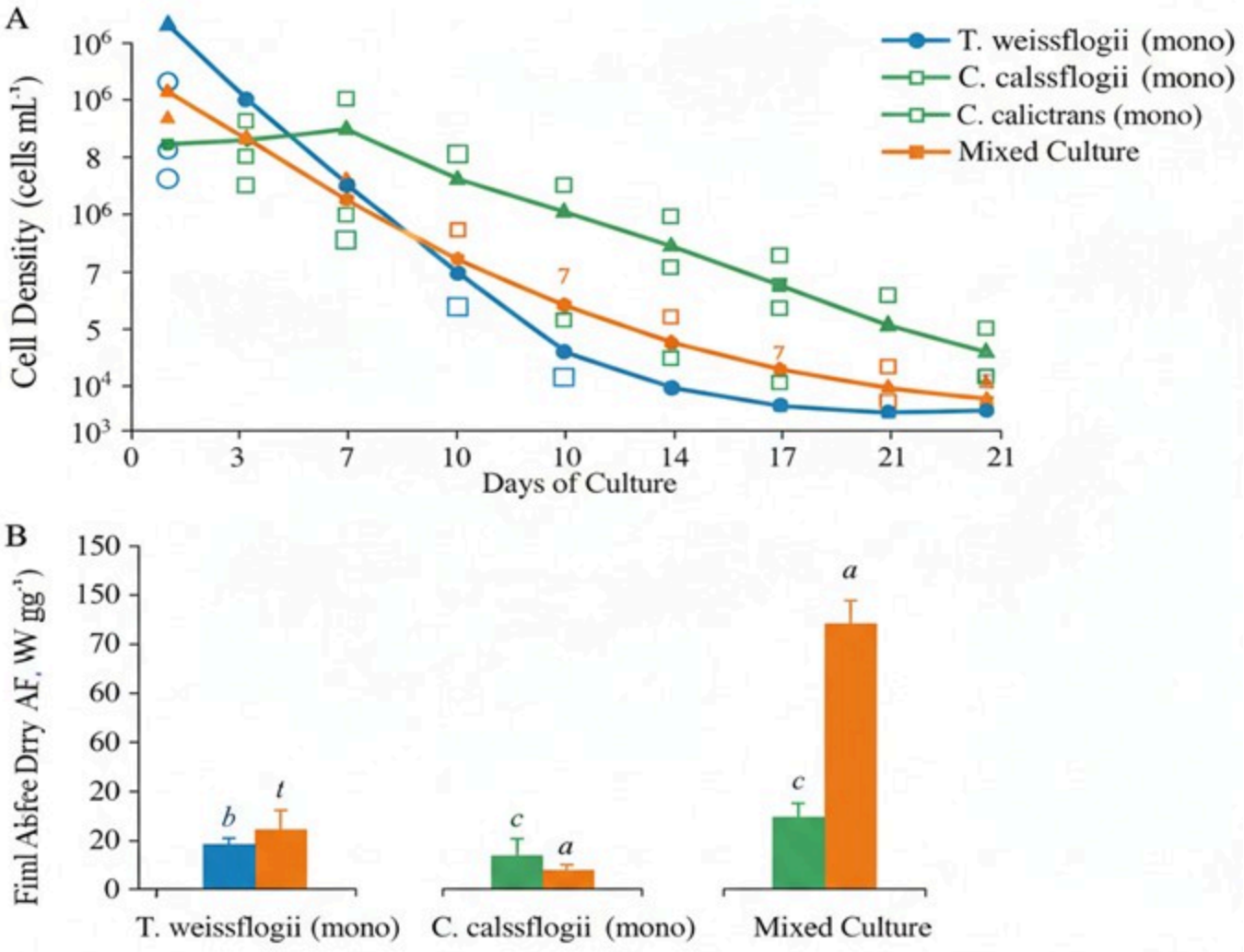
1. Abdelhay RA, El-Mor MS, Salem MAM, Al-Sagheer AA, Abd-Elhakim YM, Hassan BA, Mounes HAM (2025) Effect of nitrogen sources on diatoms growth and nutritional value for enhancing Litopenaeus vannamei larval performance. Animals 15(4):466. https://doi.org/10.3390/ani15040466
2. Achupallas JM, Zhou Y, Davis DA (2016) Pond production of Pacific white shrimp, Litopenaeus vannamei, fed grain distillers dried yeast. Aquacult Nutr 22(6):1222–1229. https://doi.org/10.1111/anu.12359
3. Association of Official Analytical Chemists (2005) Official methods of analysis. AOAC, Gaithersburg
4. Bell JG, Tocher DR, MacDonald FM, Sargent JR (1995) Diets rich in eicosapentaenoic acid and gamma-linolenic acid affect phospholipid fatty acid composition and production of prostaglandins E1, E2 and E3 in turbot (Scophthalmus maximus), a species deficient in delta 5 fatty acid desaturase. Prostaglandins Leukot Essent Fatty Acids 53(4):279–286. https://doi.org/10.1016/0952-3278(95)90128-0
5. Bell TA, Lightner DV (1988) A handbook of normal penaeid shrimp histology. World Aquaculture Society, Baton Rouge

6. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37(8):911–917. https://doi.org/10.1139/o59-099
7. Bufka J, Vaňková L, Sýkora J, Křížková V (2024) Exploring carotenoids: Metabolism, antioxidants, and impacts on human health. J Funct Foods 118:106284. https://doi.org/10.1016/j.jff.2024.106284
8. D'Alvise PW, Lillebø S, Prol-Garcia MJ, Wergeland HI, Nielsen KF, Bergh Ø, Gram L (2012) Phaeobacter gallaeciensis reduces Vibrio anguillarum in cultures of microalgae and rotifers, and prevents vibriosis in cod larvae. PLoS ONE 7(8):e43996. https://doi.org/10.1371/journal.pone.0043996
9. Debasis D, Ananda-Raja R, Ghoshal TK, Mukherjee S, Vijayan KK (2018) Evaluation of growth, feed utilization efficiency and immune parameters in tiger shrimp (Penaeus monodon) fed diets supplemented with or diet fermented with gut bacterium Bacillus sp. DDKRC1 isolated from gut of Asian seabass (Lates calcarifer). Aquac Res 49(4):2147–2155. https://doi.org/10.1111/are.13672
10. DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28(3):350–356. https://doi.org/10.1021/ac60111a017
11. González-Meza GM, Herrera-Acosta K, Casillas-Hernández R, Rentería-Mexía A, Gortáres-Moroyoqui P, Gil-Núñez JC, Ibarra-Gámez J, Ulloa-Mercado G (2022) Dietary supplementation effect of three microalgae on Penaeus vannamei growth, biochemical composition, and resistance to Vibrio parahaemolyticus (AHPND). Latin Am J Aquat Res 50(1):88–98. https://doi.org/10.3856/vol50-issue1-fulltext-2773
12. Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (eds) Culture of Marine Invertebrate Animals. Plenum, New York, pp 29–60
13. Huervana FH, Dionela CS, de la Torre EDS, del Castillo CS, Traifalgar RFM (2022) Utilization of marine diatom Thalassiosira weissflogii as a feed additive in seawater-tolerant Nile tilapia (Oreochromis niloticus, Linnaeus 1758) strain. Front Sustainable Food Syst 6:1052951. https://doi.org/10.3389/fsufs.2022.1052951
14. Jeffrey ST, Humphrey GF (1975) New spectropho-tometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochemie und Physiologie der Pflanzen 167(2):191–194. https://doi.org/10.1016/S0015-3796(17)30778-3
15. Joshi J, Srisala J, Truong VH, Chen IT, Nuangsaeng B, Suthienkul O, Thitamadee S (2014) Variation in Vibrio parahaemolyticus isolates from a single Thai shrimp farm experiencing an outbreak of acute hepatopancreatic necrosis disease (AHPND). Aquaculture 428:297–302. https://doi.org/10.1016/j.aquaculture.2014.03.030
16. Ju ZY, Forster IP, Dominy WG (2009) Effects of supplementing two species of marine algae or their fractions to a formulated diet on growth, survival and composition of shrimp (Litopenaeus vannamei). Aquaculture 292(3–4):237–243. https://doi.org/10.1016/j.aquaculture.2009.04.040

17. Katayama T, Abdu Rahman N, Khatoon H, Kasan NA, Nagao N, Yamada Y, Takahashi K, Furuya K, Wahid A, Md. Yusoff ME, Jusoh F, M (2022) Bioprospecting of tropical microalgae for high-value products: n-3 polyunsaturated fatty acids and carotenoids. Aquaculture Rep 27:101406. https://doi.org/10.1016/j.aqrep.2022.101406
18. Li Y, Xiao G, Mangott A, Kent M, Pirozzi I (2016) Nutrient efficacy of microalgae as aquafeed additives for the adult black tiger prawn, Penaeus monodon. Aquac Res 47(11):3625–3635. https://doi.org/10.1111/are.12815
19. Liao IC, Chien YH (2011) The Pacific White Shrimp, Litopenaeus vannamei, in Asia: The World's Most Widely Cultured Alien Crustacean. In: Galil B, Clark P, Carlton J (eds) the Wrong Place - Alien Marine Crustaceans: Distribution, Biology and Impacts. Springer, Dordrecht, pp 489–519. https://doi.org/10.1007/978-94-007-0591-3_17
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193(1):265–275
21. Monteiro M, Lavrador AS, Santos R, Rangel F, Iglesias P, Tárraga M, Couto A, Serra CR, Tafalla C, Da Costa E, Domingues MR, Oliva-Teles A, Carvalho AP, Enes P, Díaz-Rosales P (2021) Evaluation of the potential of marine algae extracts as a source of functional ingredients using zebrafish as animal model for aquaculture. Mar Biotechnol 23(4):529–545. https://doi.org/10.1007/s10126-021-10044-5
22. Mustonen AM, Nieminen P (2023) Dihomo-γ-linolenic acid (20:3n-6)-metabolism, derivatives, and potential significance in chronic inflammation. Int J Mol Sci 24(3):2116. https://doi.org/10.3390/ijms24032116
23. Obaldo LG, Divakaran S, Tacon AG (2002) Method for determining the physical stability of shrimp feeds in water. Aquac Res 33(5):369–377. https://doi.org/10.1046/j.1365-2109.2002.00681.x
24. Pérez-Sánchez T, Mora-Sánchez B, Balcázar JL (2018) Biological approaches for disease control in aquaculture: advantages, limitations and challenges. Trends Microbiol 26(11):896–903. https://doi.org/10.1016/j.tim.2018.05.002
25. Pettersen AK, Turchini GM, Jahangard S, Ingram BA, Sherman CDH (2010) Effects of different dietary microalgae on survival, growth, settlement and fatty acid composition of blue mussel (Mytilus galloprovincialis) larvae. Aquaculture 309(1–4):115–124. https://doi.org/10.1016/j.aquaculture.2010.09.024
26. Shah MR, Lutzu GA, Alam A, Sarker P, Chowdhury MK, Parsaeimehr A, Daroch M (2018) Microalgae in aquafeeds for a sustainable aquaculture industry. J Appl Phycol 30(1):197–213. https://doi.org/10.1007/s10811-017-1234-z
27. Tacon AGJ, Metian M, McNevin AA (2021) Future feeds: suggested guidelines for sustainable development. Reviews Fisheries Sci Aquaculture 30(2):135–142. https://doi.org/10.1080/23308249.2020.1860474
28. Zhang L, Liao K, Shi P, Guo J, Xie F, Xu J (2025) Dietary inclusion of microalgae meal for Pacific white shrimp (*Litopenaeus vannamei*): effects on growth performance, flesh quality, and immunity.

Figures

A Figure 1. Growth dynamics of Thalassisira weisslow and Chaetculture and mixed (1:1) culture. (A) Temporal changes in cell density over 21 days.



(B) Final biomass yield measured as ash-free dry weight at period. Values are presentean > SD (n=3). Different lowreclats ioceclase letters letters indicate significant differences ($p < 05$).

Figure 1

See image above for figure legend

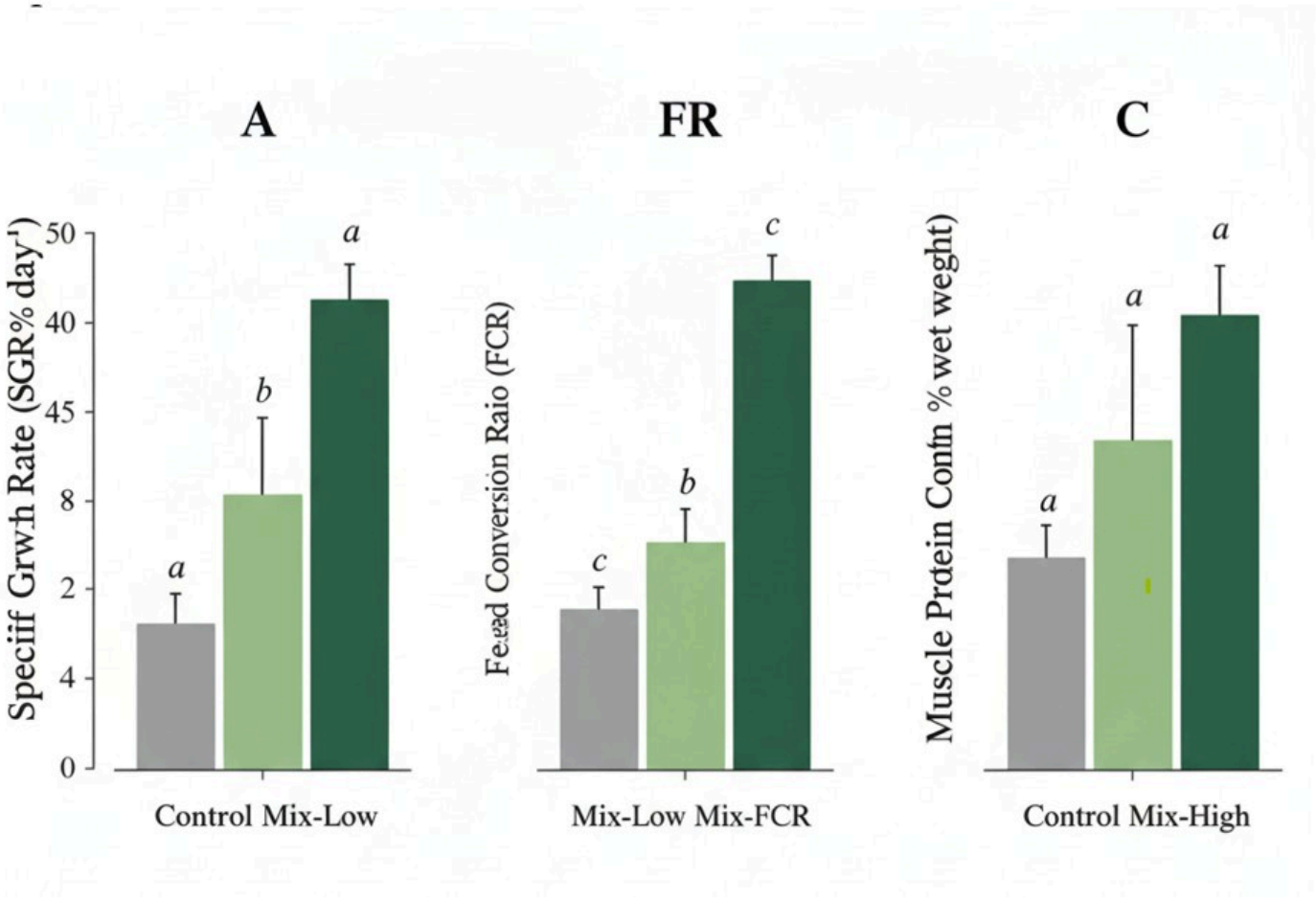


Figure 2

Effect of dietary mixed microalgae supplementation on key performance parameters of *Penaeus vannamei*. (A) Specific Growth Rate (SGR), (B) Feed Conversion Ratio (FCR), and (C) Muscle Protein Content. Shrimp were fed a control diet or diets containing 1% (Mix-Low) or 3% (Mix-High) mixed diatom biomass for 60 days. Values are presented as mean ± SD (n=3). Bars with different lowercase letters are significantly different ($p < 0.05$).

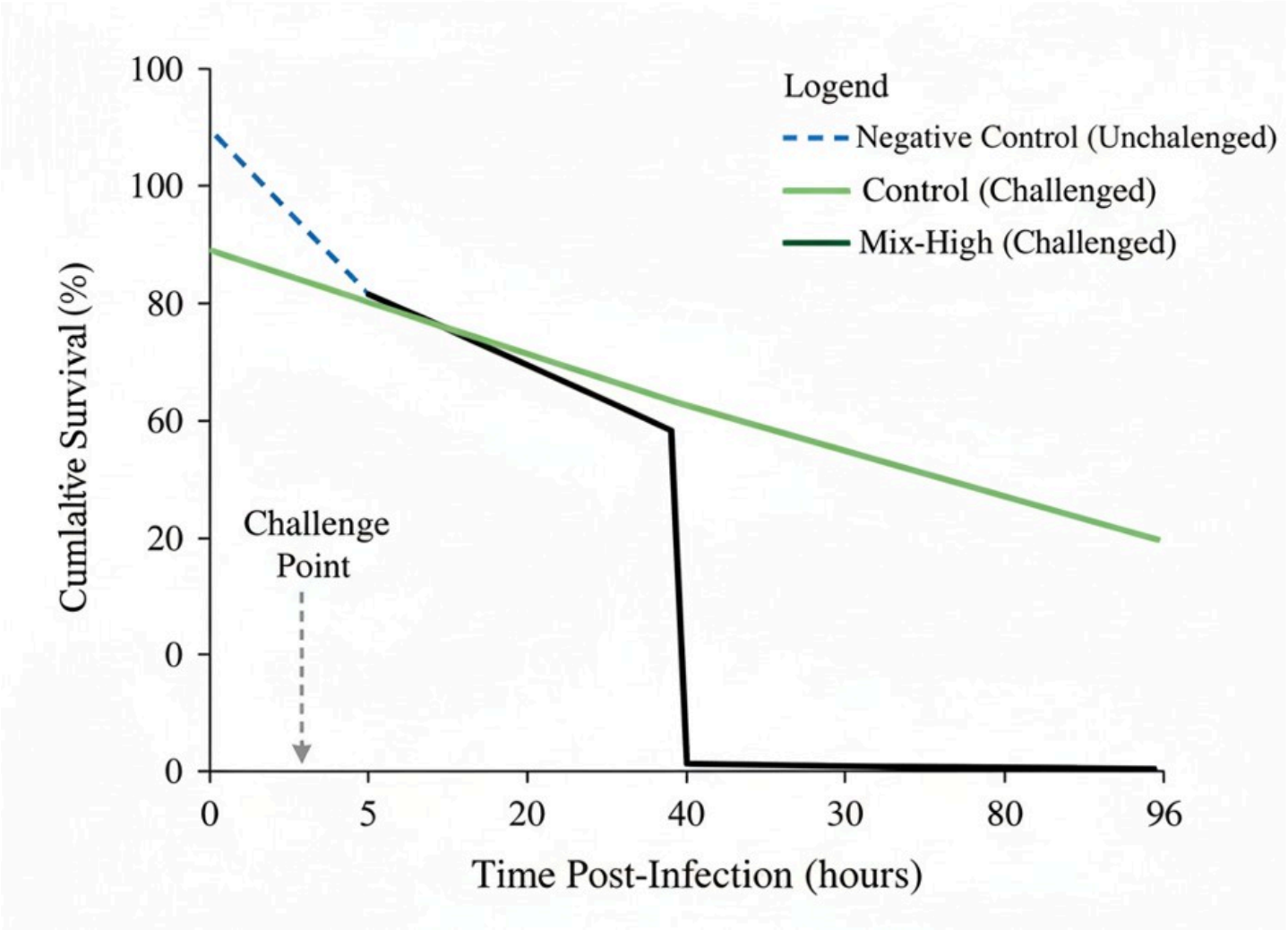


Figure 3

Survival of *Penaeus vannamei* following immersion challenge with *Vibrio parahaemolyticus* (AHPND strain). Shrimp were fed experimental diets for 60 days prior to challenge. The negative control group was not exposed to the pathogen. The arrow indicates the time of challenge. Survival in the Mix-Low and Mix-High groups was significantly higher ($p < 0.05$, Log-rank test) than in the challenged control group.

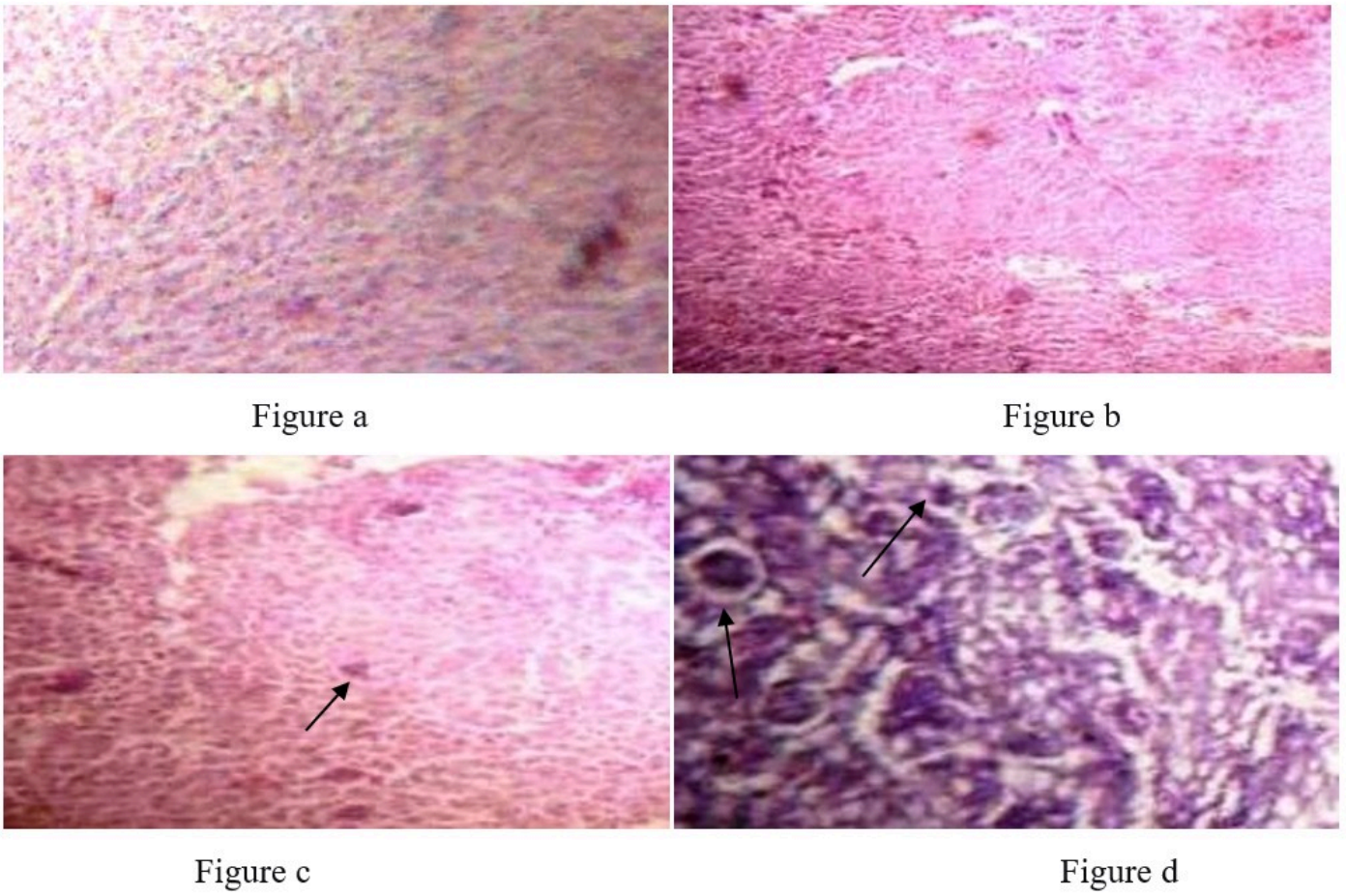


Figure 4

Histological sections of *Penaeus vannamei* hepatopancreas after *V. parahaemolyticus* challenge (H&E stain). (A, B) Shrimp fed the Mix-Low and Mix-High diets, respectively, showing normal hepatopancreatic tubule architecture with intact epithelial (E) and B-cells (B). (C, D) Shrimp fed the control diet exhibiting severe AHPND pathology, characterized by massive sloughing of tubule epithelial cells (SL) and hemocytic nodules (HN).