



Evaluation of Dietary Supplementation of Algae *Spirulina platensis* on Growth Performance and Disease Resistance in White Leg Shrimp Challenge Study against *Vibrio parahaemolyticus*

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ABSTRACT

Background: A 60-day feeding trial was conducted to evaluate the effect of dietary *Spirulina platensis* (SP) on feed utilization, growth performance and disease resistance in *Penaeus vannamei* with *Vibrio parahaemolyticus*.

Methods: *S. platensis* incorporated into isoproteic diets (35±0.5% crude protein) by partially replacing corn flour at inclusion levels of 5%, 10% and 15%, with a basal diet as the control. Juvenile shrimp (initial weight 1.15±0.08 g) were fed these experimental diets for 60 days.

Result: Results indicated that shrimp fed SP-supplemented diets showed significant improvements ($P<0.05$) in final body weight, weight gain, specific growth rate, feed conversion ratio and survival rate compared to the control. The 10% SP diet resulted in the highest crude protein and ash content and significantly lower crude lipid levels in the whole-body composition ($P<0.05$). Additionally, shrimp fed the 10% SP diet exhibited the highest survival rate ($P\leq 0.05$) and enhanced immune response while challenged with *V. parahaemolyticus*. These findings suggest that dietary inclusion of *S. platensis* at 10% optimally enhances growth performance and improves disease resistance in *P. vannamei*.

Key words: Immune parameters, *Penaeus vannamei*, *Spirulina platensis*, *Vibrio parahaemolyticus*.

INTRODUCTION

Shrimp farming has grown rapidly and emerged as one of the most significant global aquaculture industries. The Pacific whiteleg shrimp, *P. vannamei*, is the most commonly farmed species among all penaeid shrimps, accounting for over 70% of global shrimp farming production (Ashour *et al.* 2024). The application of Recirculating aquaculture system, IMTA and biofloc systems supports efficient and high-yield aquaculture production (Ezhilmathi *et al.*, 2023). Recirculating aquaculture constitutes an environmentally sustainable and health-oriented approach to modern aquaculture. This method offers several advantages, including a reduced physical footprint, water conservation capabilities, efficient operational management and elevated yield potential (Xu *et al.*, 2025). It facilitates enhanced growth performance, increased production efficiency per unit area and labor and allow for precise control of growth rates, leading to consistent and predictable harvests. A study conducted by Moses *et al.* (2024) using a recirculating aquaculture system found that adding 0.8% of a mineral mix (calcium and phosphorus) optimized the growth, biochemical and hematological parameters of Asian seabass. Shrimp producers at the grow-out stage have suffered significant financial losses in recent years due to the emergence of a bacterial disease known as acute hepatopancreatic necrosis disease (AHPND). AHPND is linked to a unique strain of *V. parahaemolyticus* and may also involve other *Vibrio* species with pVA-type plasmids. Infected shrimp show symptoms like anorexia and lethargy, leading to death from hepatopancreas

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damage. Key histological features include sloughing of epithelial cells from the hepatopancreas (Ng *et al.*, 2024).

S. platensis is a notable microalgae used in aquatic animal feeds, valued for its growth-enhancing properties. It is rich in nutrients, containing up to 65% protein, essential vitamins, amino acids, minerals and fatty acids (Zahan *et al.*, 2024). Various forms of *Arthrospira platensis* supplements, such as dry powder, whole-liquid extract,

nanoparticle derivatives and lipid-free biomass, can be included in the diets of shrimp *P. vannamei* (Ashour *et al.*, 2024). Ahmed *et al.* 2025 observed that *P. vannamei* fed with diets that included 4–8 g/kg of *S. platensis* significantly improved growth, feed efficiency, survival rates, antioxidant enzyme activity and higher resistance to *V. parahaemolyticus*. Using dried SP as a supplement has been shown to notably influence growth measures, survival rates and reproductive success in different shrimp species, such as *P. vannamei* (Sandeep *et al.*, 2023) and *Megalobrama amblycephala* (Jiang *et al.*, 2022). The incorporation of 3% and 6% *S. platensis* powder and 0.3% purified spirulina polysaccharides (PSP) into the diets of *Micropterus salmoides* resulted in better growth rates, protein efficiency and lower lipid levels, as well as increased antioxidant activity (Zhang *et al.*, 2024).

Eissa *et al.* (2024) found that dietary supplementation with *S. platensis* (5 g/kg) and curcumin nanoparticles (30 mg/kg) significantly improved the growth and health of Nile tilapia fingerlings during a 56-day trial. Dietary supplementation of *A. platensis* enhances immunological responses and growth in *P. vannamei* (Ashour *et al.*, 2024). Li *et al.* (2022) reported improved physiological health and growth from dried algae in the diet. Yudiati *et al.* (2024) noted that *Spirulina sp.* and alginate increased survival rates to 80% in postlarvae, with *Spirulina* at 9 mg/kg significantly enhancing growth ($\alpha = 0.05$). Sivakumar *et al.* (2018) found that replacing fishmeal with 42.8% *S. platensis* improved growth and survival in *P. monodon*, indicating its potential as a partial fishmeal substitute.

In light of the aforementioned benefits, the present study was initiated to investigate the effects of *S. platensis* on the growth performance, feed intake, overall body composition of *P. vannamei* reared in RAS, particularly with the pathogenic *V. parahaemolyticus*.

MATERIALS AND METHODS

Ethical statement

The experiment was conducted following the procedures of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment and Forests (Animal Welfare Division), Govt. of India on care and use of animals in scientific research. This study was approved by the ethical committee of Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India. The experiment was conducted for 60 days at Mariculture Research Farm Facility (MRFF), Tharuvaikulam, Thoothukudi, TamilNadu, India.

Experimental design, feeding and setup

The study was conducted at the Mariculture Research Farm Facility (MRFF), Tharuvaikulam, using SPF *P. vannamei* seeds sourced from KKG CeSA, Kanyakumari. After one month of rearing in a raceway system, 240 juveniles (initial weight 1.15 ± 0.08 g) were randomly distributed into 12 FRP tanks (20 shrimp/tank) with a capacity of 160 litre under Recirculatory Aquaculture System, with four dietary

treatments (T0 = 0%, T1 = 5%, T2 = 10%, T3 = 15% of SP and three replicates each. Shrimp were fed isonitrogenous diets (36.5% protein) prepared at CMFRI, Thoothukudi, three times daily (10:00 am, 1:00 pm, 4:00 pm) for 60 days at 10% body weight until satiation. Amino acid composition was analyzed via UPLC following Ishida *et al.* (1981) at TNJFU, Chennai (Table 1 and 2).

Water quality parameters

Water quality parameters, including temperature, pH, dissolved oxygen, alkalinity, salinity, ammonia-N, nitrite-N and nitrate-N, were measured weekly in RAS during the feeding trial following the standard procedures described in APHA (2005).

Growth performance and feed efficiency

In RAS, sampling was done every ten days to observe shrimp growth and modify the feeding rate as necessary. The following formulas were used to analyse the shrimp growth parameters, including weight gain percentage (WG%), total biomass, protein efficiency ratio (PER), feed conversion ratio (FCR), feed conversion efficiency (FCE) and specific growth rate (SGR).

$$\text{Weight gain (WG)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{WG\%} =$$

$$\frac{[\text{Final body weight (g)} - \text{Initial body weight (g)}]}{\text{Initial body weight (g)}} \times 100$$

$$\text{SGR} =$$

$$\frac{\ln (\text{Final body weight}) - \ln (\text{Initial body weight})}{\text{Feeding trial days}} \times 100$$

$$\text{FCR} = \frac{\text{Total amount of the feed consumed (g)}}{\text{Weight gained (g)}}$$

$$\text{PER} = \frac{\text{Wet weight gain}}{\text{Total protein given}}$$

$$\text{Survival rate} = \frac{\text{Final number of shrimp}}{\text{Initial number of shrimp}}$$

Proximate analysis shrimp body

For each group, five shrimp were chosen to examine the proximal body composition of the shrimp. The techniques described in the AOAC guidelines were used to evaluate the proximate analysis, which included measurements of crude protein (CP), moisture, crude lipid (CL) and ash. The Kjeldahl method (AOAC, 1990) was used to quantify the crude protein (CP) content, while the Salam (1994) dry extraction approach was used to estimate the crude lipid (CL) content. Samples of dried shrimp muscles were heated for seven hours at 500°C in a Muffle furnace to determine the amount of ash present. The crucible was weighed once before it was burned and once more after it was cooled. The whole shrimp were placed in a pre-weighed aluminium foil tray and dried in an electric oven set to 65°C to 80°C until a consistent weight was reached in order to determine the water content of the shrimp.

Immune challenge against *Vibrio parahaemolyticus*

After 60 days of culture, 15 shrimp per tank were selected for a 15-day challenge with *V. parahaemolyticus*. All treatment and control groups were injected with the pathogen, while an additional 15 shrimp in the control group were injected with sterile saline to serve as the

experimental control (EC). The challenge was conducted using a live bacterial suspension (10^6 CFU mL⁻¹), following Liuxy *et al.* (1996), administered intramuscularly between the third and fourth abdominal segments. Feeding continued as usual throughout the challenge period. Cumulative mortalities were recorded daily for 15 days and

Table 1: Ingredient composition and proximate analysis of the experimental diets (% on DM basis) for *P. vannamei* containing different inclusion of *S. platensis*.

Ingredients	Inclusion (%)			
	Control (0%)	Treatment 1 (5%)	Treatment 2 (10%)	Treatment 3 (15%)
Fish meal	23	23	23	23
Shrimp head meal	9	9	9	9
Soybean meal	33	28	23	18
Spirulina	0	5	10	15
Corn flour	15	10	5	0
Fish oil	4	4	4	4
Wheat flour	6.1	6.1	6.1	6.1
Broken rice	3	3	3	3
Cassava	5.5	5.5	5.5	5.5
DCP	0.2	0.2	0.2	0.2
Vit C	0.2	0.2	0.2	0.2
Mineral mix	0.5	0.5	0.5	0.5
Vitamin mix	0.5	0.5	0.5	0.5
Proximate composition				
Moisture (%)	10.45	10.46	10.46	10.24
Protein (%)	36.36	36.32	36.66	36.24
Fat (%)	4.91	4.09	4.23	4.03
Fibre(%)	2.64	2.62	2.47	2.31
Ash(%)	7.81	7.98	7.29	7.71
NFE (%)	37.83	38.53	38.89	39.47

Table 2: Amino acid profile (% of the diet) of the tested diets containing different levels of *S. platensis*.

	Control (0%)	Treatment 1 (5%)	Treatment 2 (10%)	Treatment 3 (15%)
Essential amino acids				
Arginine	2.52	2.38	2.44	2.60
Histidine	1.10	1.16	1.20	1.28
Isoleucine	2.70	2.64	2.77	2.83
Leucine	2.96	2.90	2.70	2.34
Lysine	2.15	2.36	2.50	2.44
Methionine	0.75	0.76	0.74	0.70
Phenylalanine	1.92	1.91	1.90	1.89
Threonine	1.47	1.51	1.54	1.61
Tryptophan	0.31	0.32	0.36	0.30
Valine	2.86	2.90	1.92	1.98
Non-essential amino acids				
Alanine	1.65	1.72	1.70	1.59
Aspartic acid	3.99	4.24	4.46	4.90
Glutamic acid	6.24	6.34	6.38	6.42
Glycine	1.83	1.92	2.00	2.15
Taurine	0.02	0.02	0.02	0.03
Cystine	0.54	0.53	0.53	0.53
Serine	1.81	1.83	1.86	1.90
Tyrosine	1.39	1.38	1.37	1.35

dead shrimp were promptly removed and disposed of safely. Haemolymph was collected from the base of third abdominal segment in a 1 ml syringe with 26-gauge needle size which contained 100 µl of anticoagulant solution (100 mM glucose, 30 mM trisodium citrate, 26 mM citric acid, 510 mM NaCl and 10 mM EDTA.Na2: pH = 7.3) (Vargas-albores *et al.*, 1993) on 1st, 3rd, 5th day for immune parameter analysis.

Total haemocyte count (THC)

In a micro centrifuge tube A 100 µL sample of haemolymph was collected and mixed with an equal volume of haemolymph fixative (10% formalin in 0.45 M NaCl), then allowed to stand for 10 minutes. After that, approximately 20 µL of this fixed haemocyte suspension was transferred to a new microcentrifuge tube containing the same volume of Rose Bengal solution (1.2% Rose Bengal in 50% ethanol) for haemocyte staining and this solution for 20 minutes was left to incubate at room temperature. The total haemocytes were counted in 5/25 squares on an improved Neubauer cell counting chamber (with a volume of squares equal to $0.2 \times 0.2 \times 0.1 \text{ mm}^3$) in triplicate (Raja *et al.*, 2012).

Prophenoloxidase (ProPO) activity

The activity of proPO was assessed using spectrophotometric methods (in triplicate) to observe the conversion of L-dihydroxyphenylalanine (L-DOPA) to dopachrome (Gollas-Galvan *et al.*, 1999). The cell suspension (100 µL) was incubated with 50 µL of laminarin (1 mg/ ml in cacodylate buffer) or with only cacodylate buffer for the control, for 1 hour at 20°C before adding 50 µL L-DOPA (3 mg/mL in cacodylate buffer). Following incubation, 800 µL of cacodylate buffer was added and the reaction proceeded for 10 minutes at 20°C. Absorbance at 490 nm was then measured using an ELISA reader (BioTek, USA).

Respiratory burst activity (RBA)

The respiratory burst activity was evaluated using the procedures described by Anderson and Siwiki (1995). A haemolymph sample of approximately 100 µL was combined with an equal amount of 0.2% NBT solution and left to incubate at room temperature for 30 minutes. Following this, a haemolymph suspension of 50 µL NBT was mixed with 1 mL of N, N-dimethyl formamide (DMF) and then centrifuged at 5,000 rpm for 5 minutes using an

Eppendorf 5810 R centrifuge. The resulting supernatant was separated and the absorbance was measured at 540 nm.

Superoxide dismutase (SOD) activity

To evaluate SOD activity (Campa-Cordova *et al.*, 2002), the haemolymph sample (40 µL) was mixed with 10 volumes of phosphate-buffered saline (PBS) and then centrifuged (Eppendorf 5810R) at 6000 g, 4°C for 7 minutes. Following centrifugation, the supernatant was heated in a water bath at 65°C for 5 minutes to obtain the SOD crude extract. Next, 150 µL of SOD crude extract was combined with 50 µL of Nitro blue tetrazolium (NBT) reagent and left to incubate for 2 minutes. The optical density was then gauged at 630 nm using an ELISA reader from BioTek, USA.

Catalase activity

To evaluate catalase activity (Takahara *et al.*, 1960), approximately 2.5 mL of phosphate buffer and 0.5 µL of haemolymph were combined and the enzyme reaction was started by adding 1.0 mL of hydrogen peroxide solution. The alterations in absorbance at 240 nm were observed every 30 seconds for up to 3 minutes to measure the enzyme activity.

Statistical analysis

Data were analyzed using IBM SPSS 26. The Shapiro-Wilk test assessed normality (N = 40). Two-way ANOVA evaluated the effects of two variables on weight among T1, T2, T3 and Control, with significant differences identified ($P < 0.001$). Tukey's post hoc test was performed ($P < 0.05$) for multiple comparisons. One-way ANOVA examined differences in shrimp bio-growth and composition, with results presented as mean $\pm$ SD.

RESULTS AND DISCUSSION

Growth performance and feed utilization

Shrimp fed with spirulina-supplemented diets exhibited significantly improved growth performance compared to the control group, as shown in Table 3. Final weight ranged from 8.21 ± 0.15 g (control) to 10.82 ± 0.08 g (Treatment 2), with Treatment 2 showing significantly higher growth parameters than all other treatments ($p < 0.05$; Tukey's HSD post hoc test applied). Weight gain, specific growth rate (SGR) and protein efficiency ratio (PER) were also highest

Table 3: Growth performance and feed efficiency of *P. vannamei* fed with *S. platensis*-supplemented diets over 60 days.

Parameters	Control	Treatment 1	Treatment 2	Treatment 3
Initial weight	1.51 ± 0.01	1.53 ± 0.015	1.52 ± 0.010	1.52 ± 0.015
Final weight	8.21 ± 0.15^d	10.12 ± 0.15^b	10.82 ± 0.08^a	9.57 ± 0.05^c
Weight gain (g)	6.70 ± 0.20^d	8.59 ± 0.03^b	9.3 ± 0.07^a	8.05 ± 0.43^c
Weight gain (%)	442.96 ± 3.03^d	559.05 ± 7.50^b	611.84 ± 2.35^a	527.31 ± 3.97^c
SGR	2.82 ± 0.009^d	3.14 ± 0.018^b	3.27 ± 0.005^a	3.06 ± 0.010^c
FCR	2.24 ± 0.06^a	1.74 ± 0.002^c	1.61 ± 0.01^d	1.86 ± 0.11^b
PER	0.18 ± 0.004^d	0.23 ± 0.000^b	0.25 ± 0.002^a	0.22 ± 0.001^c
Survival rate (%)	95.02 ± 0.05	96.12 ± 0.01	98.51 ± 0.02	95.34 ± 0.01

in Treatment 2, indicating superior feed utilization efficiency. The feed conversion ratio (FCR) was most efficient in Treatment 2 (1.61 ± 0.01), significantly lower than the control ($p < 0.05$). Survival rates across all groups were high (95.02%-98.51%) with no significant differences ($p > 0.05$). These results suggest that spirulina inclusion up to 10% enhances shrimp growth while maintaining survival, potentially through improved nutrient utilization and feed efficiency.

Proximate analysis shrimp body

Dietary spirulina supplementation significantly improved the nutritional composition of *P. vannamei*, as shown in Table 4. Shrimp in Treatment 2 (10% spirulina) exhibited the highest dry matter ($24.34 \pm 0.23\%$) and crude protein ($23.51 \pm 0.45\%$) contents, both significantly higher than the control and other treatments ($p < 0.05$; Tukey's HSD). Crude fat content was also significantly higher in Treatment 2 ($9.88 \pm 0.32\%$), followed by Treatment 1. The highest ash content ($2.73 \pm 0.85\%$) was observed in Treatment 3, suggesting potential mineral retention at higher spirulina inclusion. Moisture content (74.32%-75.45%) showed no significant differences across treatments ($p > 0.05$). These findings indicate that spirulina improves protein and lipid deposition in shrimp muscle, potentially enhancing flesh quality and energy storage, which are important for growth and immunity.

Water quality parameter

Water quality parameters remained within optimal ranges for *P. vannamei* throughout the experiment, as shown in Table 5. Temperature (28.10 - 28.22°C) and salinity (31.9-32.2 ppt) showed no significant differences ($p > 0.05$). However, dissolved oxygen and pH were significantly higher in Treatment 2 (5.41 ± 0.12 mg/L and 7.82 ± 0.27 , respectively) compared to the control ($p < 0.05$), likely due to enhanced microbial activity from spirulina supplementation. Ammonia, nitrate and nitrite levels were significantly lower in spirulina-fed groups, with

Treatment 2 showing the lowest values, indicating improved water quality and nitrogen utilization ($p < 0.05$).

Immune parameters

Total haemocyte count

On Day 1, total haemocyte count (THC) values were uniformly low across all groups (EC, PC, T1, T2, T3), with no statistically significant differences ($p > 0.05$), indicating a baseline immune status at the start of the experiment (Fig 1). By Day 3, a significant increase in THC was observed across all groups, with the highest counts recorded in Treatment 1 (T1) and Treatment 2 (T2). The THC values in these groups were significantly higher compared to both Day 1 and Day 5 ($p < 0.05$), suggesting a marked immune activation in response to spirulina supplementation. By Day 5, THC values declined from the Day 3 peak but remained significantly elevated compared to Day 1 ($p < 0.05$). At this point, no significant differences were detected among the treatment groups, indicating a potential return to immune equilibrium.

Respiratory burst activity

Respiratory burst activity showed no significant differences among groups on Day 1 ($p > 0.05$), suggesting similar baseline oxidative responses across treatments. By Day 3, a significant reduction in respiratory burst activity was observed in all groups compared to Day 1 and Day 5 ($p < 0.05$). This reduction may reflect a temporary suppression of respiratory burst mechanisms following initial immune stimulation. By Day 5, respiratory burst activity increased again and returned to levels comparable to Day 1, indicating recovery or normalization of immune function, as shown in Fig 2.

Prophenoloxidase activity

Prophenoloxidase activity was initially high in all groups on Day 1, with no significant differences among treatments ($p > 0.05$). By Day 3, a significant decline in activity was noted

Table 4: Whole-body proximate composition (%) of *P. vannamei* fed diets with varying levels of *S. platensis*.

Parameters	Control	Treatment 1	Treatment 2	Treatment 3
Dry matter (%)	22.15 ± 0.26^d	23.75 ± 0.4^b	24.34 ± 0.23^a	22.54 ± 0.32^c
moisture	75.12 ± 0.12^a	74.53 ± 0.18^a	75.45 ± 0.15^a	74.32 ± 0.10^a
Protein	19.52 ± 0.55^d	22.64 ± 0.48^b	23.51 ± 0.45^a	22.31 ± 0.50^c
Fat	7.44 ± 0.25^d	9.56 ± 0.22^b	9.88 ± 0.32^a	8.72 ± 0.24^c
Ash	1.82 ± 0.88^d	2.50 ± 0.74^c	2.54 ± 0.75^b	2.73 ± 0.85^a

Table 5: Water quality parameters in the rearing tanks of Pacific white shrimp over 60 days Mean ($\pm$ SD, n = 15).

Parameters	Control	Treatment 1	Treatment 2	Treatment 3
Temperature	28.10 ± 0.14	28.22 ± 0.12	28.15 ± 0.13	28.19 ± 0.11
Dissolved oxygen	5.14 ± 0.15	5.36 ± 0.14	5.41 ± 0.12	5.29 ± 0.13
pH	7.60 ± 0.28	7.75 ± 0.26	7.82 ± 0.27	7.70 ± 0.25
Salinity(ppt)	32.0 ± 0.30	32.1 ± 0.28	31.9 ± 0.25	32.2 ± 0.27
NH ₃ (mgL ⁻¹)	0.066 ± 0.007	0.058 ± 0.006	0.053 ± 0.005	0.061 ± 0.006
NO ₃ (mgL ⁻¹)	0.75 ± 0.02	0.68 ± 0.03	0.65 ± 0.02	0.70 ± 0.02
NO ₂ (mgL ⁻¹)	0.45 ± 0.15	0.38 ± 0.12	0.35 ± 0.14	0.40 ± 0.13

across all groups ($p < 0.05$), though levels remained higher than those observed on Day 5. On Day 5, ProPO activity further decreased significantly to its lowest point ($p < 0.05$). A significant interaction between treatment groups and sampling days was observed, indicating that the decline in ProPO activity was influenced by both treatment and time. These results suggest that ProPO activity undergoes a time-dependent reduction post-stimulation, possibly due to regulatory immune feedback mechanisms (Fig 3).

Superoxide dismutase (SOD) activity

Superoxide dismutase activity was lowest on Day 1 across all groups ($p > 0.05$), indicating a baseline antioxidant status, as shown in Fig 4. By Day 3, SOD activity significantly increased in all groups, with Treatment 2 exhibiting the highest activity ($p < 0.05$), suggesting an upregulation of antioxidant defences in response to spirulina supplementation. This trend continued on Day 5, where SOD activity remained significantly elevated, particularly in Treatment 2, demonstrating

a sustained antioxidant response that may contribute to improved immune resilience.

Catalase (CAT) activity

Catalase activity was highest across all groups on Day 1 ($p < 0.05$), indicating an immediate oxidative stress response, as shown in Fig 5. By Day 3 and Day 5, CAT activity significantly decreased in all groups ($p < 0.05$), with no significant differences among treatments at these later time points. The observed decline suggests a time-dependent reduction in oxidative stress, possibly reflecting physiological adaptation or effective antioxidant regulation induced by spirulina supplementation.

Cumulative mortality

Following *V. parahaemolyticus* challenge, the pathogen control (PC) group exhibited the highest cumulative mortality (50%). In contrast, shrimp fed spirulina-supplemented diets showed reduced mortality: 40% in

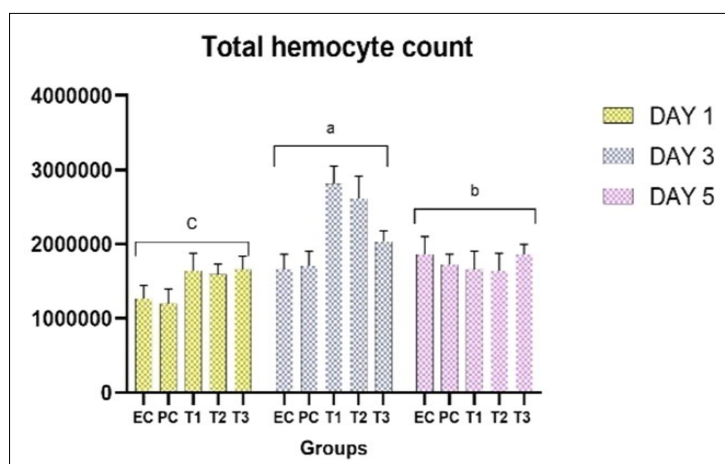


Fig 1: Total hemocyte count (THC) in the recirculating aquaculture system (RAS) showed significant differences across treatment groups and time intervals.

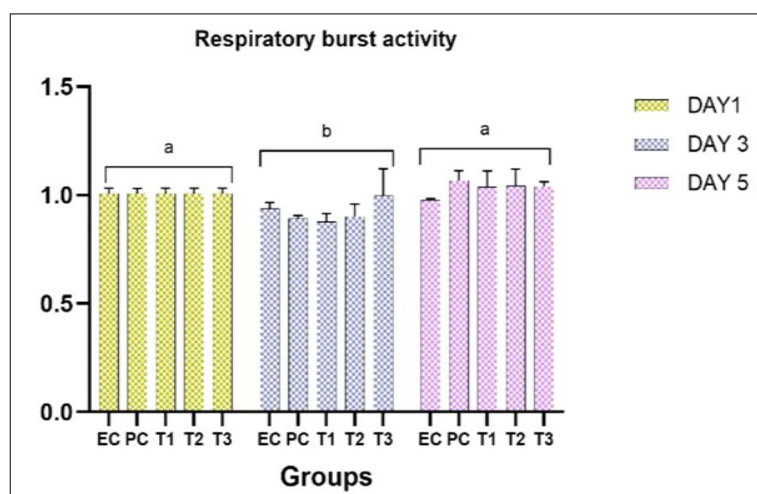


Fig 2: Respiratory burst activity displayed significant variation across different time intervals, with a notable interaction between treatment groups and sampling days.

Treatment 1 and 30% in both Treatment 2 and Treatment 3, with mortality stabilizing in Treatment 3 after Day 9. No mortality was observed in the unchallenged experimental control (EC). These findings suggest that dietary *S. platensis*, particularly at higher inclusion levels, enhances disease resistance and improves survival outcomes in *P. vannamei* as shown in Fig 6.

Growth performance

The present study investigated the effects of dietary *S. platensis* supplementation at 0%, 5%, 10% and 15% inclusion levels on the growth performance and survival of *P. vannamei*. The results revealed that the 10% inclusion level (T2) significantly improved final weight, weight gain, specific growth rate (SGR) and protein efficiency ratio (PER), while reducing feed conversion ratio (FCR), indicating optimal utilization of nutrients. The findings are consistent with the research conducted by Teimouri *et al.* (2013), which indicated that replacing fish meal with up to 10% *Spirulina*

platensis (SP) significantly enhances the growth performance of rainbow trout. Similarly, replacing fishmeal with 10% *S. platensis* meal in *Lates calcarifer* diets improved growth, feed efficiency, gut enzyme activity and immune-antioxidant responses. The 10% inclusion is recommended for better performance and health in juveniles (Mousavi *et al.*, 2025).

Li *et al.* (2022) observed that the inclusion of dried algae as a feed supplement significantly enhanced feed intake (FI), improved feed conversion ratio (FCR), elevated carcass quality and supported better growth metrics. moreover, it contributed to an improved physiological condition during adverse circumstances, such as stress and disease. SP is high in Polyunsaturated fatty acids (PUFA), especially the n-3 and n-6 varieties that are essential for the synthesis of biomembranes (Vigliano Relva *et al.*, 2024). These blue-green microalgae improve the intestinal flora of shrimp by breaking down indigestible feed components and increasing the production of enzymes that assist in fat metabolism (Jaime-Ceballos *et al.*, 2006;

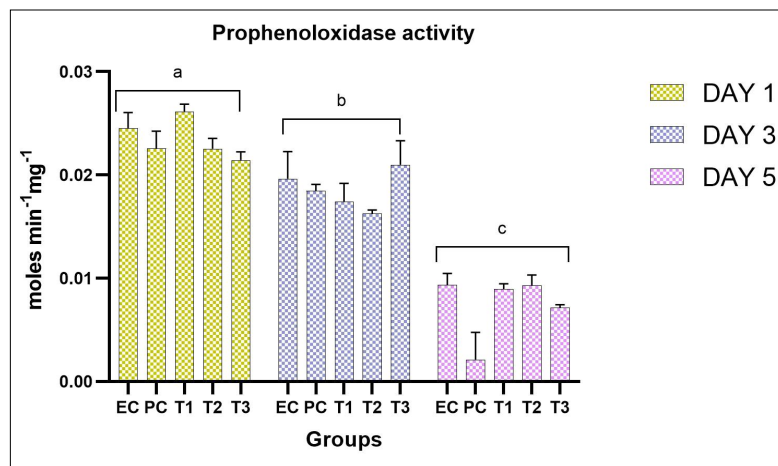


Fig 3: Prophenoloxidase activity showed significant differences across treatment groups and time intervals.

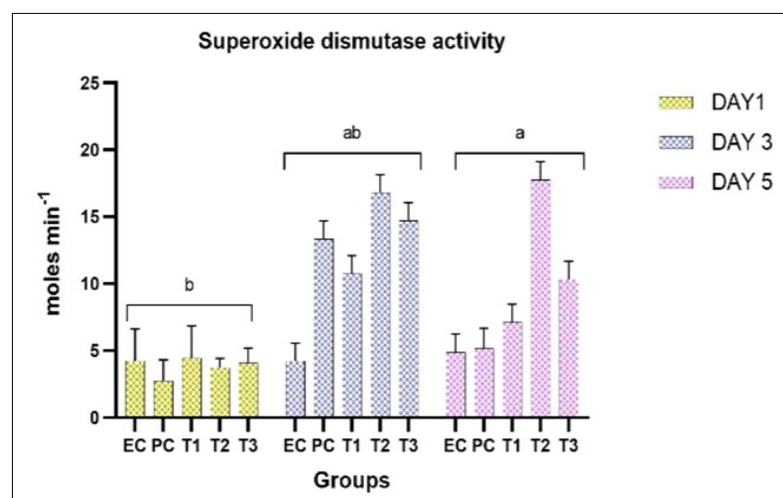


Fig 4: Superoxide dismutase (SOD) activity showed significant differences across treatment groups and time intervals.

Sandeep *et al.*, 2023). SP enhances growth and feed efficiency by increasing the population of beneficial bacteria in the gastrointestinal tract (Jaime-Ceballos *et al.*, 2006). Likewise, in the present study, spirulina-fed shrimp showed better growth performance than the control.

Moreover, the survival rate was highest in the T2 group, suggesting that Spirulina may contribute to immune enhancement. Studies have demonstrated that Spirulina exerts immunomodulatory effects by stimulating phagocytic activity and enhancing resistance to bacterial infections such as vibriosis (Al-Ghanayem, 2023). In the present study, a 15% inclusion level (T3) showed improvements over the control group but did not exceed the performance of the 10% group. This suggests a plateau effect at higher inclusion levels, supported by earlier studies indicating that excessive dietary Spirulina may negatively impact palatability or nutrient balance (Li *et al.*, 2022).

Whole body proximate composition

Dietary inclusion of *S. platensis* significantly influenced the proximate composition of *P. vannamei*. In the present study, a 10% inclusion level (T2) produced the highest protein ($23.51 \pm 0.45\%$) and fat ($9.88 \pm 0.32\%$) contents. The results are consistent with the earlier research conducted by Hossain *et al.* (2017) and Sukri *et al.* (2016). The increased ash content in the 15% group ($2.73 \pm 0.85\%$) may be attributed to Spirulina rich mineral composition, which includes calcium, magnesium and iron, essential for shrimp growth and metabolism (Abdelnour *et al.*, 2020). Moisture levels remained consistent across treatments, indicating no adverse effect of Spirulina on tissue hydration. These results are consistent with earlier findings that Spirulina improves body composition and feed quality in aquatic species when included at optimal levels (Wang *et al.*, 2025).

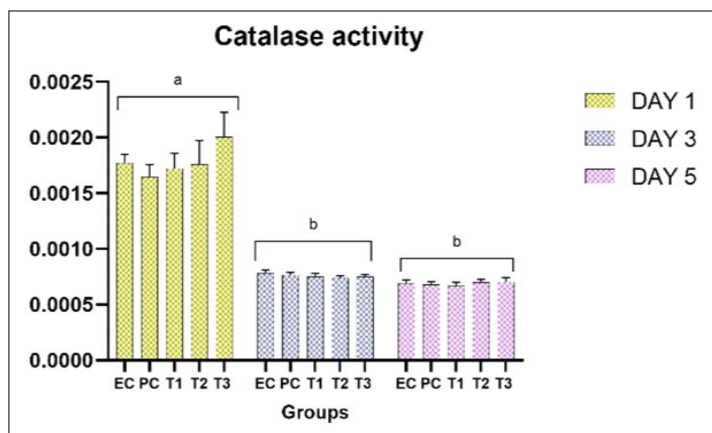


Fig 5: The catalase (CAT) activity in the RAS (Recirculating Aquaculture System) varied significantly across different time intervals and treatment groups.

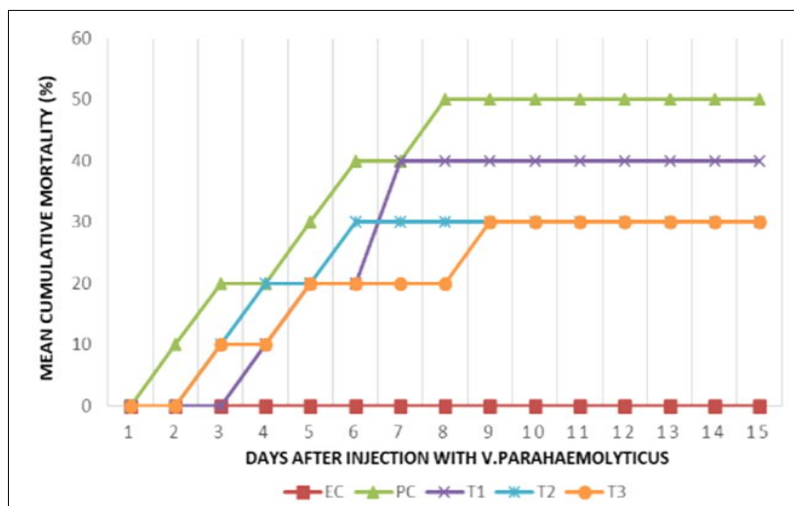


Fig 6: Cumulative mortality of *P. vannamei* challenged with *V. parahaemolyticus* and fed diets supplemented with varying levels of *S. platensis*.

Immune response

This study evaluated the immunomodulatory effects of dietary *S. platensis* at varying inclusion levels (5%, 10% and 15%) in *P. vannamei* over a 60-day feeding period, followed by a pathogenic challenge with *V. parahaemolyticus*. The results of the current study showed that the incorporation of dietary dried *S. platensis* supplements significantly enhanced resistance to the pathogenic bacteria *V. parahaemolyticus*.

P. vannamei fed with Spirulina-supplemented diets (T1, T2, T3) demonstrated a significant enhancement in total hemocyte count (THC), particularly on Day 3, when compared to the control groups (EC and PC), indicating a strengthened cellular immune response. Respiratory burst activity, which is essential for the production of reactive oxygen species involved in pathogen elimination, was also significantly higher in Spirulina-fed groups, especially on Days 3 and 5. Additionally, prophenoloxidase (PO) activity, a key component of the shrimp immune defense, was elevated on Day 1 in the Spirulina-fed groups and remained consistently higher than the control groups throughout the study period. Superoxide dismutase (SOD) activity, which plays a critical role in antioxidant defense, was also significantly upregulated in shrimp receiving Spirulina, suggesting improved management of oxidative stress during pathogenic challenges. These results align with the findings of Tayag *et al.* (2010), who reported that hot-water extracts of *S. platensis* enhanced innate immunity in *P. vannamei*, as evidenced by increased hemocyte counts, immune enzyme activities and resistance to *V. alginolyticus* following both injection and immersion treatments. Similarly, Chen *et al.* (2016) demonstrated that dietary supplementation with dried Spirulina powder significantly boosted lysozyme activity and improved shrimp resistance to *V. alginolyticus* infections, further supporting Spirulina role as an effective immunostimulant in shrimp aquaculture.

Overall, the results of this study demonstrate that dietary Spirulina, particularly at 10% inclusion (T2), can effectively enhance both antioxidant and immune responses in *P. vannamei*, thereby improving resilience against *V. parahaemolyticus* infection.

CONCLUSION

The present study demonstrates that dietary inclusion of *S. platensis* significantly enhances the growth performance, feed efficiency and disease resistance of *P. vannamei*. Among the tested levels, a 10% inclusion of *S. platensis* proved most effective, resulting in improved growth parameters, optimal whole-body composition and the highest survival rate following *V. parahaemolyticus* challenge. These findings suggest that *S. platensis* can be a promising functional feed supplementation in shrimp aquaculture, contributing to sustainable production through enhanced growth and immune responses.

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Conflict of interest

Every step taken was compliant with the responsible parties' ethical standards and the authors declare that they have no conflict of interest.

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