

Effect of dietary C-Phycocyanin on growth, survival, haematology, immune response, gut microbiome and disease resistance of Pacific white shrimp, *Penaeus vannamei*

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Abstract

C-Phycocyanin (CPC) was extracted from *Arthrospira* sp. and isolated from an estuarine ecosystem in southern India. A 45-day feeding trial was conducted to unravel the effect of dietary CPC in *Penaeus vannamei*. Seven iso-nitrogenous and iso-energetic experimental diets were prepared with different levels of CPC at 0 (CPC₀), 50 (CPC₅₀), 100 (CPC₁₀₀), 200 (CPC₂₀₀), 400 (CPC₄₀₀), 800 (CPC₈₀₀) and 1600 (CPC₁₆₀₀) mg per kg diet. The results revealed that the survival percentages in CPC₂₀₀, CPC₄₀₀, CPC₈₀₀ and CPC₁₆₀₀ were significantly ($p < 0.05$) higher than in other treatments. Moreover, *P. vannamei* fed with CPC₈₀₀ and CPC₁₆₀₀ showed a significantly higher percentage ($p < 0.05$) of weight gain compared to other treatments. Similarly, compared to other treatments, average body weight, average daily gain, specific growth rate and feed conversion ratio improved significantly ($p < 0.05$) in CPC₈₀₀ and CPC₁₆₀₀. Differential gene expression of selected immune genes (*ProPO*, *SOD* and *HSP70*) indicated enhanced immunity in *P. vannamei* fed with CPC₄₀₀ and higher CPC doses. The enhanced health of *P. vannamei* fed with diets containing CPC was further corroborated by the gut *Vibrio* counts, haematology, gut microbiome and histopathological analyses, and the results showed the addition of CPC at 800 mg per kg of feed enhanced the health of shrimps. The results also give the baseline information about the utilization of CPC in *P. vannamei* grow-out feed at an inclusion level of 800 mg per kg of feed to get better growth, survival and health. The results would help in developing functional feeds for shrimps to act against bacterial diseases.

KEYWORDS

Arthrospira, C-Phycocyanin, immunity, shrimp aquaculture

1 | INTRODUCTION

Aquaculture is one of the fastest growing food-producing sectors in the world for the past many years. Shrimp is the major commodity in terms of economic value in aquaculture production. More than 90% of the global shrimp production is contributed by a single species, *Penaeus vannamei* (FAO, 2020). The ever-increasing demand for quality protein as well as the profit from shrimp farming is attracting the farmers to go for intensive farming. However, the frequent disease outbreaks remain a challenge for sustainable shrimp aquaculture production. The causative agents of infectious diseases in shrimp farming are mainly viruses and bacteria (Ananda Raja et al., 2017). Some *Vibrio* spp. are pathogenic contributing to several epizootic diseases which are of serious concern in shrimp culture and at times responsible for mass mortalities of shrimp in commercial aquaculture (Ananda Raja et al., 2017; de Souza Valente & Wan, 2021; Ina-Salwany et al., 2019).

As an alternative to the use of antibiotics, studies have recommended the usage of immunostimulants in feeds to improve the immune responses to control the diseases in aquaculture with supporting evidence for their beneficial role in aquaculture (Hassaan et al., 2021; Prabu et al., 2018; Wang et al., 2017). Dietary supplementation of immunostimulants served a dual purpose of enhancing production as well as providing nutritional and health benefits. Thus, the use of feed containing beneficial additives that enhances health and immunity is the need of the hour to minimize disease occurrence and stress to the shrimps (Barman et al., 2013).

The blue-green algae, spirulina, is often regarded as a superfood and has wide application in different fields like human nutrition, animal nutrition, aquaculture, pharmaceuticals, etc., mainly due to the presence of several vital nutrients such as protein, amino acids, vitamins, pigments and bioactive compounds in high quality and quantity (Belay et al., 1996; Ghaeni & Roomiani, 2016; Sandeep et al., 2015). The nutraceutical properties of spirulina were evident from the enhanced physiological, immunological and anti-oxidative status of farmed species fed with spirulina supplemented diets (Abdel-Tawwab & Ahmad, 2009; Al-Deriny et al., 2020; Chen et al., 2016; Macias-Sancho et al., 2014).

C-Phycocyanin (Phycocyanin from cyanobacteria, CPC), a water-soluble pigment, is one of the major pigment constituents of spirulina (Boussiba & Richmond, 1980; Erikson, Eriksen, 2008). This biologically active phytochemical compound is a portion of the phycobiliprotein of spirulina (Boussiba & Richmond, 1980) and has potent antioxidant activity capable of enhancing immunity, growth and productivity in aquaculture (Hassaan et al., 2021). Various properties of CPC such as anti-oxidant, anti-inflammatory, hepatoprotective and nephroprotective properties have been well documented (de Jesus Raposo et al., 2013; Fernández-Rojas et al., 2014; Romay et al., 2003; Sitohy et al., 2008). Previous research works have indicated CPC to act as a nutraceutical compound and dried spirulina as a natural feed additive or as supplements for enhancing the growth (Abd-El Alim et al., 2018; Al-Deriny et al., 2020). However,

the detailed study of its applications in aquaculture nutrition and health management is limited. The stimulatory effect of dietary CPC on survival and disease resistance was also reported in guppy, *Poecilia reticulata* (Biabani Asrami et al., 2019) and Asian seabass larvae (Gora et al., 2019). Furthermore, the growth-promoting and immunostimulatory effects of CPC were reported in Nile tilapia, *Oreochromis niloticus* (El-Araby et al., 2022; Hassaan et al., 2021). However, scientific studies on the effect of dietary phycocyanin on penaeid shrimps, where bacterial infections are more prevalent and critical, are lacking. The immunomodulatory effect of CPC has been studied (Ashaolu et al., 2021; Omar et al., 2022) and proven in fish (El-Araby et al., 2022; Hassaan et al., 2021; Muchtar et al., 2019). The present study explored the possible effects of dietary CPC on the immune genes of *P. vannamei*, by evaluating the relative gene expression of *ProPO*, *SOD* and *HSP70* before and after challenging (AC) with *Vibrio campbellii*. Any dietary addition of bioactive compounds or extracts can alter the gut microbiome of shrimp (Mariot et al., 2021). The present study details the gut microbiology and gut microbiome to better understand the changes happening in the shrimp gut at the microbial level by the dietary addition of CPC. Histology is an excellent tool for understanding the cellular changes in shrimps fed with bioactive compounds (Jahromi et al., 2021). Hence, the present study was conducted to understand the effects of dietary CPC on the growth performance, survival, haematological parameters, immune response, gut microbiome and disease resistance of *P. vannamei* challenged with *Vibrio campbellii* and to evaluate their potential application in shrimp aquaculture.

2 | MATERIALS AND METHODS

2.1 | Ethical statement

The experiments in the present study were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, a statutory committee of the Department of Animal Husbandry and Dairying, Ministry of Fisheries, Animal Husbandry and Dairying, Govt. of India. Further, the approval was obtained from the Institutional Animal Ethics Committee of the ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India.

2.2 | Isolation of *Arthrospira* sp. from the estuarine ecosystem

Water samples were collected from Muttukadu estuarine area, Tamil Nadu, India (12°48'37.4"N and 80°14'42.8"E). The samples were serially diluted in the modified NRC (Nallayan Research Centre) medium (NaHCO_3 : 8 g/L, NaCl : 5 g/L, NaNO_3 : 2.5 g/L, K_2SO_4 : 0.5 g/L, K_2HPO_4 : 0.5 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.16 g/L, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$: 0.01 g/L) as described in Sandeep et al. (2015). After 2 weeks of incubation in an indoor microalgae laboratory (24 ± 1°C; 3000 lux; 16:8 Light:Dark),

test tubes with cell growth were streaked in agar plates enriched with modified NRC medium. The isolation protocol was followed as described previously (Andersen & Kawachi, 2005; Sandeep et al., 2018). The plates with colonies were re-streaked 4–5 times to get the single colony. Colonies obtained through repeated streaking were transferred using a sterile loop from the agar plates into liquid-modified NRC medium in 100ml conical flasks for further growth (Figure 1). The liquid cultures were kept at $24 \pm 1^\circ\text{C}$; 3000lux; 16:8 L:D. Identification of *Arthrospira* sp. was done based on morphological characteristics observed under the microscope (Nikon Ci-I Eclipse).

2.3 | Scanning electron microscopy

The protocol for scanning electron microscopy (SEM) was followed as described by Kumar et al. (2022). *Arthrospira* sp. biomass was fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer at 4°C for 8 h and dehydrated in ethanol series (50%, 70%, 90%, 100%) for 15 min each followed by chemical drying with 100% ethanol and 100% hexamethyldisilazane (HMDS) in 3:1, 1:1 and 1:3 composition for 15 min each and final overnight drying of the sample in 100%

HMDS. The samples were observed under a scanning electron microscope JSM IT 300LV (JEOL).

2.4 | Biomass production of *Arthrospira* sp. and extraction of CPC

The pure culture of *Arthrospira* sp. was sub-cultured in a modified NRC medium under photoautotrophic conditions. In the modified medium, urea and phosphoric acid of NRC medium were replaced by sodium nitrate (2.5 g/L) and di-potassium hydrogen phosphate (0.5 g/L), and also, the concentration of ferrous sulphate heptahydrate was reduced from 0.5 to 0.01 g/L (Sandeep et al., 2015). The biomass was harvested in the log phase of the culture from 20L carboys and freeze-dried (SCAN VAC). The freeze-dried biomass was used for CPC extraction. The phycocyanin was extracted by Repeated Freezing and Thawing of cells in 50mM sodium phosphate buffer at a pH of 6.8, followed by ammonium sulphate treatment and estimation (Siegelman & Kycia, 1978). The purity of phycocyanin extracted was calculated in terms of the ratio of optical densities at A_{620}/A_{280} (Boussiba & Richmond, 1979). The percentage yield and the purity of CPC extracted from *Arthrospira* sp. was compared with

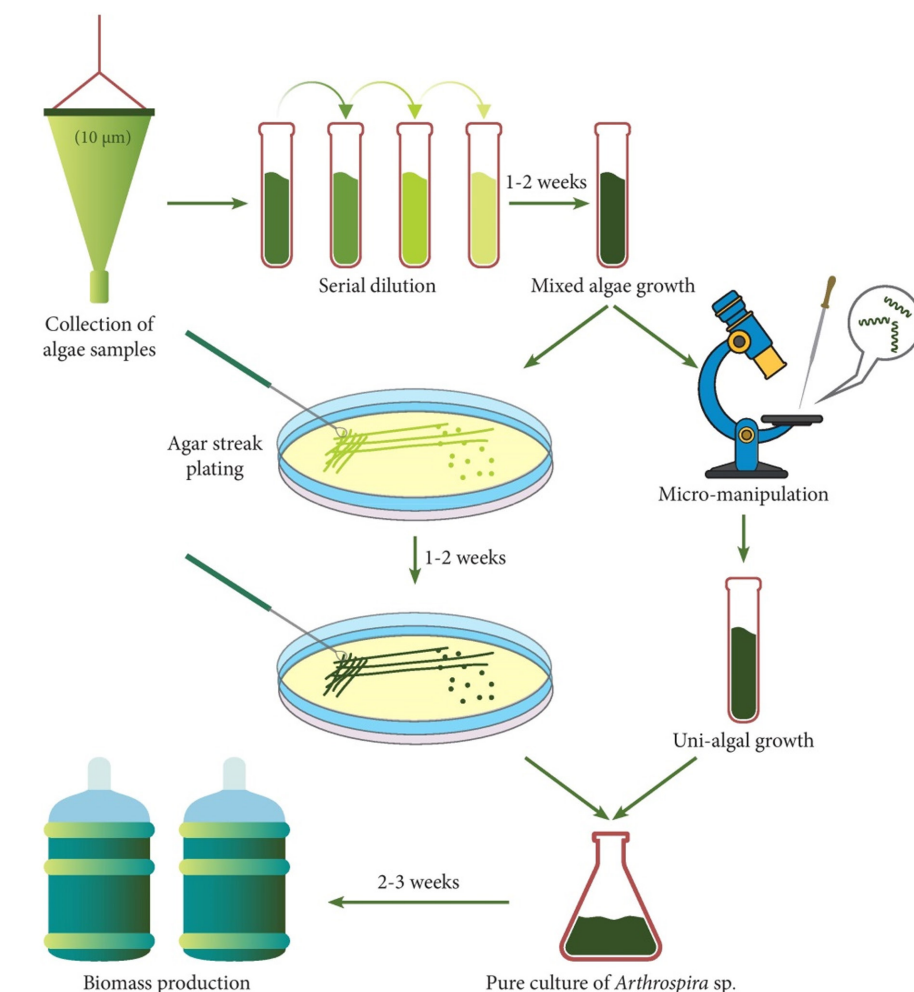


FIGURE 1 Diagrammatic representation of isolation of *Arthrospira* sp. from bloom.

commercial spirulina powder (*Arthrospira platensis*) in three different stages of extraction.

2.5 | Experimental diet

Seven iso-nitrogenous and iso-energetic experimental diets were prepared with different levels of CPC supplementation at 0 (control CPC₀), 50 (CPC₅₀), 100 (CPC₁₀₀), 200 (CPC₂₀₀), 400 (CPC₄₀₀), 800 (CPC₈₀₀) and 1600 (CPC₁₆₀₀) mg per kg diet. As there is no published literature on the effect of dietary CPC on penaeid shrimps, the doses of CPC were selected based on the previous studies on the effect of CPC on Nile tilapia, *Oreochromis niloticus* (El-Araby et al., 2022; Hassaan et al., 2021), Guppy, *Poecilia reticulata* (Biabani Asrami et al., 2019) and Asian seabass, *Lates calcarifer* (Gora et al., 2019). This was further validated using dietary spirulina studies on penaeid shrimps (Hajiahmadian et al., 2012; Pakravan et al., 2017; Silva-Neto et al., 2012). The ingredients used and the chemical composition of the experimental diets are shown in Table 1. The dry solid ingredients were ground separately into a fine powder and filtered through a 100-µm sieve and further homogenized. A sufficient quantity of water was added to this mash to make moist dough. The dough was steam-cooked and cooled. Then, vitamin and mineral mixtures were added to this dough. This was homogenized again for uniformity.

2.6 | Analysis of the proximate composition of feed

The proximate composition of experimental diets such as moisture, crude protein, crude lipid, ash, crude fibre was analysed according to the standard methods of AOAC (2005).

2.7 | Experimental animals and feeding trial

Juveniles of *P. vannamei* with average body weight (ABW) 3.99 ± 0.11 g were distributed into 21 fibre reinforced plastic tanks (100 L water each) in a recirculatory aquaculture system at 15 animals per tank in Muttukadu Experimental Station of ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India. Each treatment in the experiment was conducted in triplicate following a completely randomized design. The experimental setup was regularly monitored and maintained for standard water quality parameters among the different treatments. During the trial period, the feeding was done three times daily for visual satiation. Then, feeding was adjusted according to the daily feed consumption in each tank.

2.8 | Growth performances

All the animals were individually weighed at the beginning of the experiment; bulk weight was calculated fortnightly interval to calculate

the feeding ration. At the end of 45 days experiment, all the animals were individually weighed. ABW, total biomass, percentage weight gain (WG %), specific growth rate (SGR), average daily gain (ADG), survival and feed conversion ratio (FCR) were estimated using the formulae mentioned by Steffens (1989).

Percentage of weight gain (%) =

$$\{(\text{Mean final weight} - \text{Mean initial weight}) / \text{Mean initial weight}\} \times 100$$

Average daily gain (mg/day) =

$$(\text{Mean final weight} - \text{Mean initial weight}) / \text{days}$$

Specific growth rate (%/day) =

$$[(\ln \text{ final weight} - \ln \text{ initial weight}) / \text{days}] \times 100$$

Survival rate (%) =

$$(\text{Final number of shrimp} / \text{Initial number of shrimp}) \times 100$$

$$\text{Feed conversion ratio (FCR)} = \text{Total feed} \frac{\text{consumed}}{\text{shrimp}} \text{ weight gain}$$

2.9 | Haematology

After 45 days of feeding trial, haemolymph (0.1 ml) was collected from the animals through the ventral sinus of the first abdominal segment for estimating the total haemocyte count (THC), granular haemocyte (GH) and nongranular haemocyte (NGH) counts (Ananda Raja et al., 2017). GH and NGH counts were taken as per the method described by Ananda Raja et al. (2017). The proportions of GH that included both large granular haemocytes (LGH) and small granular haemocytes (SGH), and NGH which included large nongranular haemocytes (LNGH) and small nongranular haemocytes (SNGH) were calculated as per previous studies (Ananda Raja et al., 2017).

2.10 | Gut microbiology

Gut samples were collected and used for microbial analysis as per Kumar et al. (2017). At the end of the feeding trial, six animals from each treatment (randomly taken two from each replicate) were taken and anaesthetized with clove oil (50 µl/L) and transferred to ice. The aseptically removed gut was weighed and homogenized in normal saline solution (NSS; 0.89%). Then, samples were serially diluted (10-fold) in NSS and 100 µl of appropriate dilutions was plated on nutrient agar having 1.5% w/v NaCl and 2% agar concentration for total plate count and thiosulfate citrate bile salt sucrose agar for total *Vibrio* count. The plates were incubated at 30 °C for 48 h, and colonies were counted in the range of 30 to 300 and expressed as colony-forming units (CFU)/g of the gut.

TABLE 1 Ingredients used and the chemical composition of experimental diets containing different levels of dietary C-Phycocyanin (CPC)

	CPC ₀	CPC ₅₀	CPC ₁₀₀	CPC ₂₀₀	CPC ₄₀₀	CPC ₈₀₀	CPC ₁₆₀₀
Ingredients (g/kg)							
Fish meal	170	170	170	170	170	170	170
Dried acetes	80	80	80	80	80	80	80
Dry fish	50	50	50	50	50	50	50
Ground nut oil cake	40	40	40	40	40	40	40
Wheat	210	210	210	210	210	210	210
Soya	300	300	300	300	300	300	300
Fish oil	15	15	15	15	15	15	15
Soy lecithin	15	15	15	15	15	15	15
Vitamin mix ^a	10	10	10	10	10	10	10
Mineral mix ^b	10	10	10	10	10	10	10
Binder	10	10	10	10	10	10	10
Choline chloride	1	1	1	1	1	1	1
Vitamin C	1	1	1	1	1	1	1
CPC	0	0.05	0.1	0.2	0.4	0.8	1.6
Proximate composition (g/kg)							
Moisture	91.7±0.6	90.3±1.1	88.9±2.1	89.4±3.4	90.7±0.4	92.1±1.1	90.9±0.11
Crude protein	370.4±4.5	364.2±2.5	373.6±0.2.6	369.1±2.2	369.2±3.3	365.1±4.9	362.7±3.7
Ether extract	54.6±0.8	53.8±0.6	53.7±0.4	53.1±0.04	53.7±0.8	54.2±0.5	54.0±0.2
Crude fibre	22.3±0.5	21.7±0.4	26.8±0.9	25.0±0.04	26.8±0.09	21.9±0.3	21.8±0.4
Total ash	85.7±3.2	76.9±5.9	83.9±3.9	87.6±4.4	76.9±3.2	90.0±8.5	81.8±2.8
NFE	375.2±4.3	393.3±6.1	373.1±6.6	375.8±5.5	382.7±7.3	376.7±6.9	388.7±0.9

Note: ^a and ^b compositions are as per Ambasankar et al. (2022).

2.11 | Challenge study

After 45 days of the feeding trial, shrimps were challenged with a virulent strain of *Vibrio campbellii* LB10 isolated from a luminescent bacterial disease that affected shrimp hatchery and one LD₅₀ dose of this strain was equivalent to 5×10^3 CFU/shrimp (Kumar et al., 2021). Shrimp was challenged intramuscularly by 50 µl of bacterial suspension resulting in 5×10^3 CFU per shrimp. A control group received 50 µl of NSS without bacterium. All the challenged shrimps were observed for mortality for 5 days. No water was exchanged during the period. The result is presented as cumulative mortality at the end of challenge study.

2.12 | Metagenome analysis for the bacterial diversity in shrimp gut

The effects of dietary CPC on the gut microbiome of *P. vannamei* fed with experimental diets at different levels of CPC supplementation were studied. Three shrimps from each treatment were collected before and after challenge, and their gut was aseptically dissected out and fixed in 95% ethanol for DNA isolation and metagenome studies. Based on the data on growth performances, the shrimps from CPC₅₀ and CPC₁₀₀ were not considered for metagenome analysis.

2.12.1 | Bacterial DNA isolation and 16S rRNA gene amplification

The total bacterial DNA from the gut content was extracted using QIAamp Fast DNA Stool Mini Kit (Qiagen) as per the manufacturer's instructions. The quantity and purity of the extracted DNA was analysed using both NanoDrop (Nanodrop 2000 spectrophotometer; Thermo Fisher Scientific) and 1% agarose gel electrophoresis. The V3-V4 hyper-variable region of the bacterial 16S rRNA gene was amplified using the primer set (341F: 5'-CCTACGGGNGGCWGCAG-3' and 805R: 5'-GACTACHVGGGTATCTAATCC-3'). Sequencing libraries were generated using the Herculanase II Fusion DNA Polymerase Nextera XT Index Kit V2 (Illumina). The PCR products were sequenced on an Illumina MiSeq platform (~250bp paired-end reads) using bar-coded primer.

2.12.2 | Amplicon sequencing data analyses

The quality of the raw reads obtained from the sequencer was assessed using the FASTQC v0.11.8 tool. The reads were further trimmed using TRIMMOMATIC 0.39 tool. The trimmed/clean-reads file was subjected to the QIIME pipeline for further analysis. The sequence file was subject to OTU picking, phylogenetic reconstruction

and taxonomy assignment using the open referencing method against the SILVA v.132 database. The OTU table resulted from the OTU picking step was summarized to obtain the absolute and relative abundances of the taxonomy profile. The taxonomy profile was further used for downstream analysis such as alpha and beta diversity analyses, followed by visualization using R packages.

2.13 | Gene expression

Total RNA extracted from the gill tissues of both pre- and post-challenged shrimps using TRIzol Reagent (Invitrogen) was treated with RNase-free DNase I (Promega) and was reverse transcribed to the first strand cDNA using the PrimeScript™ first-Strand cDNA Synthesis Kit (Takara) with oligo(dT)12–18 primers. cDNA was used to measure the relative abundance of mRNA transcript of immune genes, namely prophenoloxidase (ProPO), superoxide dismutase (SOD) and heat shock protein 70 (HSP70) in gills of the shrimps by quantitative real-time PCR analysis using ABI StepOne Plus thermocycler with 1× Power SYBR Green Master Mix (Applied Biosystems) and gene-specific primers as reported in previous study (Tomy et al., 2016). The relative transcription value was calibrated with the internal control *ef-1α* gene (Leelatanawit et al., 2012; Zhou et al., 2018).

2.14 | Histopathology

The fixed hepatopancreas (HP) samples were dehydrated in ascending grades of alcohol and then hardened, cleared, embedded in paraffin and sectioned at 5 μm thicknesses for light microscopy and stained with haematoxylin and eosin following standard histopathological procedures (Bell & Lightner, 1988). Histopathological imaging was done using Ci-I Eclipse Microscope (Nikon) DS Fi2 camera and NIS image analysis software.

2.15 | Statistical analysis

The growth characteristics and relative gene expression were statistically analysed using the statistical package SPSS version 16.0

in which data were subjected to one-way analysis of variance. Duncan's multiple range test was used as a post hoc test to determine the significant differences between the means at 5% level of significance. Survival rates among different treatment groups after challenge with *V. campbellii* LB10 were estimated by Kaplan–Meier method, using the survival and survminer package in R4.0.2. The Venn diagram was made using the ggVenn Diagram ver.1.2.0 package, and plots for the abundance of the intestinal bacterial community were constructed using ggplot2 ver.3.3.5 package with the R language tool. Intestinal bacterial community structure was compared through the principal co-ordinates analysis (PCoA) with Bray–Curtis distances in R using the mix Omics package and the alpha diversity of bacterial assemblage including diversity (Shannon), community richness and evenness (Pielou's) were calculated using the vegan package.

3 | RESULTS

3.1 | Isolation of *Arthrospira* sp. from estuarine ecosystem

The SEM images showed that the *Arthrospira* sp. in the present study was having larger diameter and with the trichomes having no or very little constriction at cross-walls (Figure 2). On solidified media, *Arthrospira* sp. displayed higher gliding motility.

3.2 | Extraction of CPC

The percentage yield of CPC from *Arthrospira* sp. was compared with commercial spirulina powder (*Arthrospira platensis*), and there was no significant difference between the yields of CPC in three different stages of extraction (Percentage yield of CPC from commercial spirulina powder was 14.45 ± 0.56 and that of *Arthrospira* sp. used in the present study was 14.25 ± 0.62). Similarly, the purity of CPC extracted from *Arthrospira* sp. (1.14 ± 0.09) was also compared with that of commercial spirulina powder (1.23 ± 0.07) in three stages of extraction. There was no significant difference in the purity of CPC extracted from both.

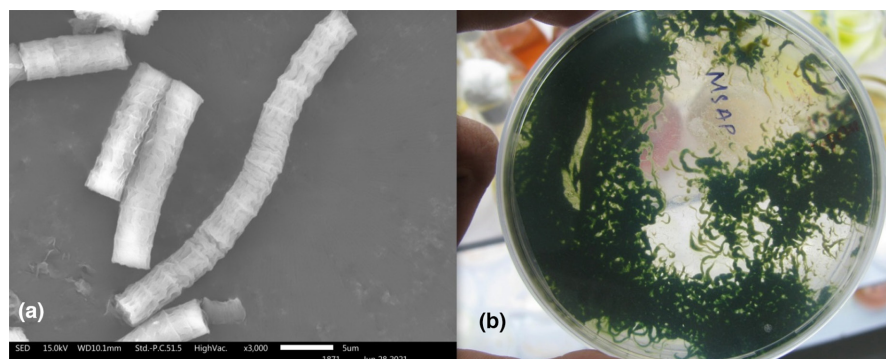


FIGURE 2 Scanning electron micrograph of *Arthrospira* sp (a) and its growth in agar plates (b).

3.3 | Feeding experiment

Penaeus vannamei fed with CPC₈₀₀ and CPC₁₆₀₀ showed significantly higher WG% ($p < 0.05$) compared to all other treatments including control (Table 2). The survival percentage in CPC₂₀₀, CPC₄₀₀, CPC₈₀₀ and CPC₁₆₀₀ was significantly ($p < 0.05$) higher than in other treatments. Similarly, WG, ABW, ADG, SGR and FCR were significantly better ($p < 0.05$) in *P. vannamei* fed with CPC₈₀₀ and CPC₁₆₀₀ compared to rest of the treatments (Table 2). It was observed that in all the above-mentioned growth parameters, there was no significant difference ($p > 0.05$) between *P. vannamei* fed with CPC₈₀₀ and CPC₁₆₀₀.

3.4 | Haematology

Total haemocyte count, SGH and LGH of *P. vannamei* significantly increased ($p < 0.05$) in the treatments fed with CPC₄₀₀ to CPC₁₆₀₀ as compared to control and other treatments (Table 3). In the case of SNGH, the values were significantly higher in the treatments fed with CPC₈₀₀ to CPC₁₆₀₀.

Percentage occurrence of haematological parameters of *P. vannamei* fed with graded level of dietary CPC showed that LGH

significantly increased in the treatments fed with CPC₂₀₀ to CPC₁₆₀₀ as compared to control and other treatments (Table 4). On the other hand, there was a significant increase in SNGH and LNGH in the shrimps fed with CPC₁₆₀₀ as compared to other treatments including control.

3.5 | Microbiology

Gut microbial load and *Vibrio* load among different treatment groups were analysed at the end of the experiment, and the results are presented in Figure 3. The total microbial load did not differ significantly among the treatments. However, *Vibrio* counts tend to reduce with increasing levels of dietary CPC. The lowest *Vibrio* count was observed at CPC₈₀₀ level which was significantly ($p < 0.05$) lower than the control group.

3.6 | Challenge study

The cumulative mortality of *P. vannamei* post challenge with *V. campbellii* LB10 is presented in Figure 4. The results suggest that treatments with higher levels of CPC levels (CPC₂₀₀

TABLE 2 Growth performance of *Penaeus vannamei* fed with graded level of dietary C-Phycocyanin (CPC)

	Survival (%)	ABW (g)	WG (g)	WG (%)	AGD (mg/day)	SGR	FCR
CPC ₀	83.33 ± 0 ^a	13.78 ± 0.40 ^a	9.88 ± 0.44 ^a	252.99 ± 14.02 ^a	0.219 ± 0.01 ^a	2.80 ± 0.09 ^{ab}	1.28 ± 0.04 ^c
CPC ₅₀	86.11 ± 4.81 ^a	14.38 ± 0.46 ^b	10.29 ± 0.51 ^{ab}	251.46 ± 15.39 ^{ab}	0.229 ± 0.01 ^{ab}	2.79 ± 0.1 ^a	1.27 ± 0.08 ^c
CPC ₁₀₀	88.89 ± 4.81 ^a	14.35 ± 0.29 ^b	10.44 ± 0.39 ^{ab}	267.57 ± 17.81 ^{ab}	0.232 ± 0.01 ^{ab}	2.89 ± 0.12 ^{ab}	1.24 ± 0.04 ^c
CPC ₂₀₀	97.22 ± 4.81 ^b	14.62 ± 0.31 ^{bc}	10.75 ± 0.43 ^b	277.59 ± 20.16 ^b	0.239 ± 0.01 ^b	2.95 ± 0.12 ^{bc}	1.24 ± 0.09 ^c
CPC ₄₀₀	97.22 ± 4.81 ^b	15.01 ± 0.34 ^c	10.92 ± 0.26 ^b	277.25 ± 2.09 ^b	0.243 ± 0.006 ^b	2.89 ± 0.01 ^{ab}	1.14 ± 0.01 ^b
CPC ₈₀₀	100 ± 0 ^b	16.19 ± 0.11 ^d	12.2 ± 0.18 ^c	305.88 ± 9.82 ^c	0.271 ± 0.004 ^c	3.11 ± 0.05 ^d	1.01 ± 0.01 ^a
CPC ₁₆₀₀	100 ± 0 ^b	16.16 ± 0.02 ^d	12.13 ± 0.06 ^c	300.81 ± 6.96 ^c	0.269 ± 0.001 ^c	3.08 ± 0.03 ^{cd}	1.05 ± 0.01 ^a

Note: Data are shown as mean ± SD ($n = 3$); mean values in the column with different superscripts differ significantly ($p < 0.05$).

Abbreviations: ABW, average body weight; ADG, average daily gain; FCR, feed conversion ratio; SGR, specific growth rate; WG, weight gain.

TABLE 3 Haematological parameters of *Penaeus vannamei* fed with graded level of dietary C-Phycocyanin (CPC)

	THC × 10 ⁷	SNGH × 10 ⁷	LNGH × 10 ⁷	SGH × 10 ⁷	LGH × 10 ⁷
CPC ₀	3.63 ± 0.21 ^b	0.70 ± 0.1 ^b	0.20 ± 0.01 ^{ab}	2.10 ± 0.2 ^b	0.67 ± 0.06 ^b
CPC ₅₀	3.77 ± 0.12 ^b	0.67 ± 0.06 ^b	0.27 ± 0.06 ^b	2.20 ± 0.1 ^b	0.67 ± 0.06 ^b
CPC ₁₀₀	2.10 ± 0.01 ^a	0.43 ± 0.06 ^a	0.17 ± 0.06 ^{ab}	1.23 ± 0.12 ^a	0.30 ± 0.01 ^a
CPC ₂₀₀	4.20 ± 0.1 ^b	0.70 ± 0.1 ^b	0.10 ± 0.01 ^a	2.57 ± 0.15 ^c	0.80 ± 0.01 ^{bc}
CPC ₄₀₀	4.87 ± 0.42 ^c	0.73 ± 0.06 ^b	0.13 ± 0.06 ^{ab}	2.90 ± 0.26 ^c	1.07 ± 0.06 ^d
CPC ₈₀₀	6.60 ± 0.5 ^d	0.90 ± 0.01 ^c	0.20 ± 0.01 ^{ab}	3.97 ± 0.25 ^d	1.60 ± 0.2 ^e
CPC ₁₆₀₀	4.97 ± 0.47 ^c	0.93 ± 0.06 ^c	0.27 ± 0.06 ^b	2.57 ± 0.12 ^c	0.87 ± 0.06 ^c

Note: Data are shown as mean ± SD ($n = 3$); mean values in the column with different superscripts differ significantly ($p < 0.05$).

Abbreviations: LNGH, large nongranular haemocyte; SGH, small granular haemocyte; SNGH, small nongranular haemocyte; THC, total haemocyte count.

	SNGH (%)	LNGH (%)	SGH (%)	LGH (%)
CPC ₀	18.83 ± 3.33 ^{bc}	5.67 ± 1.53 ^b	57.83 ± 2.52 ^{ab}	17.67 ± 0.29 ^{ab}
CPC ₅₀	17.17 ± 0.76 ^{bc}	6.67 ± 1.04 ^{bc}	59.00 ± 1.8 ^{ab}	17.17 ± 0.76 ^b
CPC ₁₀₀	19.33 ± 2.36 ^{bc}	7.67 ± 0.76 ^c	57.50 ± 2.56 ^{ab}	15.50 ± 0.5 ^a
CPC ₂₀₀	16.67 ± 2.52 ^{abc}	2.83 ± 0.76 ^a	60.83 ± 2.62 ^b	19.67 ± 0.96 ^d
CPC ₄₀₀	15.33 ± 0.76 ^{ab}	3.00 ± 0.5 ^a	59.83 ± 1.89 ^{ab}	21.83 ± 0.76 ^e
CPC ₈₀₀	13.17 ± 1.26 ^a	3.00 ± 0.5 ^a	59.50 ± 1 ^{ab}	24.33 ± 1.26 ^f
CPC ₁₆₀₀	20.17 ± 2.25 ^c	6.00 ± 0.67 ^{bc}	56.10 ± 1.01 ^a	19.00 ± 0.87 ^{cd}

Note: Data are shown as mean ± SD (n = 3); mean values in the column with different superscripts differ significantly (p < 0.05).

Abbreviations: LGH, large granular haemocyte; LNGH, large nongranular haemocyte; SGH, small granular haemocyte; SNGH, small nongranular haemocyte.

TABLE 4 Percentage occurrence of haematological parameters of *Penaeus vannamei* fed with graded level of dietary C-Phycocyanin (CPC)

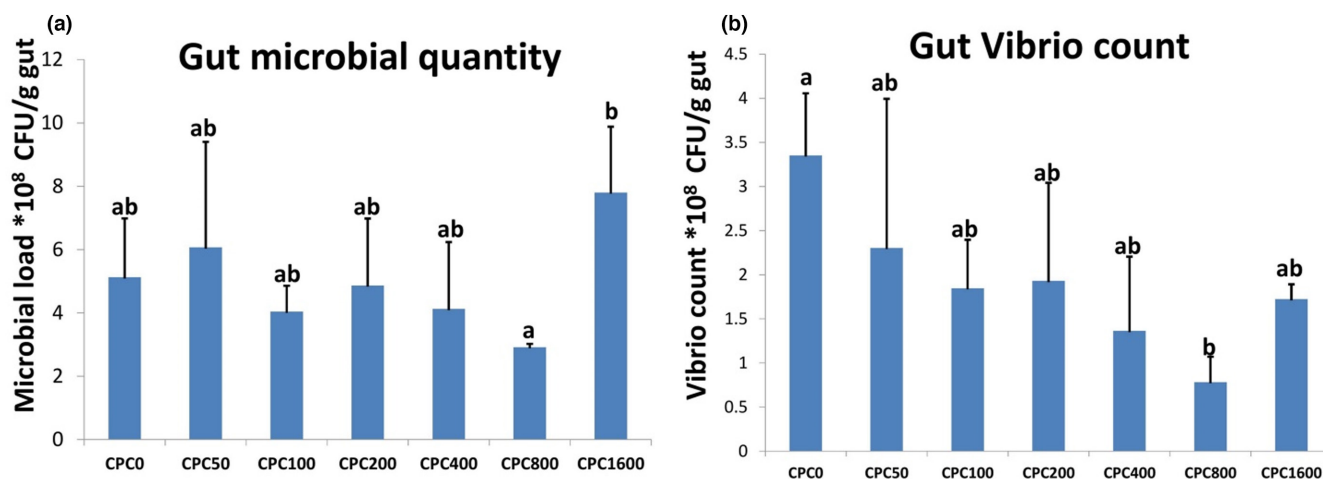


FIGURE 3 Gut microbial load (a) and *Vibrio* load (b) in *Penaeus vannamei* fed with graded levels of C-Phycocyanin (CPC). Data are shown as mean ± SD; mean values in the same bars with different alphabets differ significantly (p < 0.05).

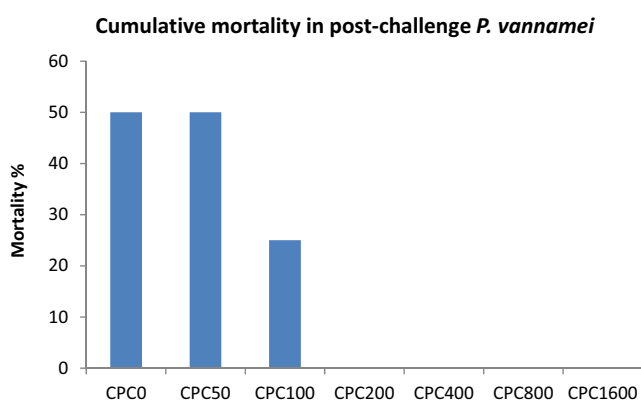


FIGURE 4 Cumulative mortality of *Penaeus vannamei* after challenge

to CPC₁₆₀₀) had 100% survival post challenge. In comparison, only 50% survival was recorded in the control (CPC₀) and CPC₅₀ groups.

3.7 | Metagenome analysis to study the bacterial diversity in shrimp gut

3.7.1 | Operational taxonomic units of intestinal bacterial communities from *P. vannamei* fed different levels of CPC and challenged with *V. campbellii* LB10

Venn diagram showing the unique and shared operational taxonomic units (OTUs) within all the pre-challenge groups (Figure 5), post-challenge groups (Figure 5b) and between pre-challenge and post-challenge groups in each CPC concentrations (Figure 5c-g) is provided in Figure 5. A total of 426 (Figure 5) and 560 (Figure 5b) bacterial genera were identified by 16S rRNA gene sequencing, respectively, from the gut of pre-challenged and post-challenged *P. vannamei* fed with different levels of CPC. More OTUs (84 OTUs; 20%) were found to be shared by all the pre-challenged groups, whereas a greater number of OTUs in the post-challenge group were observed in the control feed, CPC₀ (168 OTUs; 30%). Increase in bacterial genera was observed in the post-challenge groups in

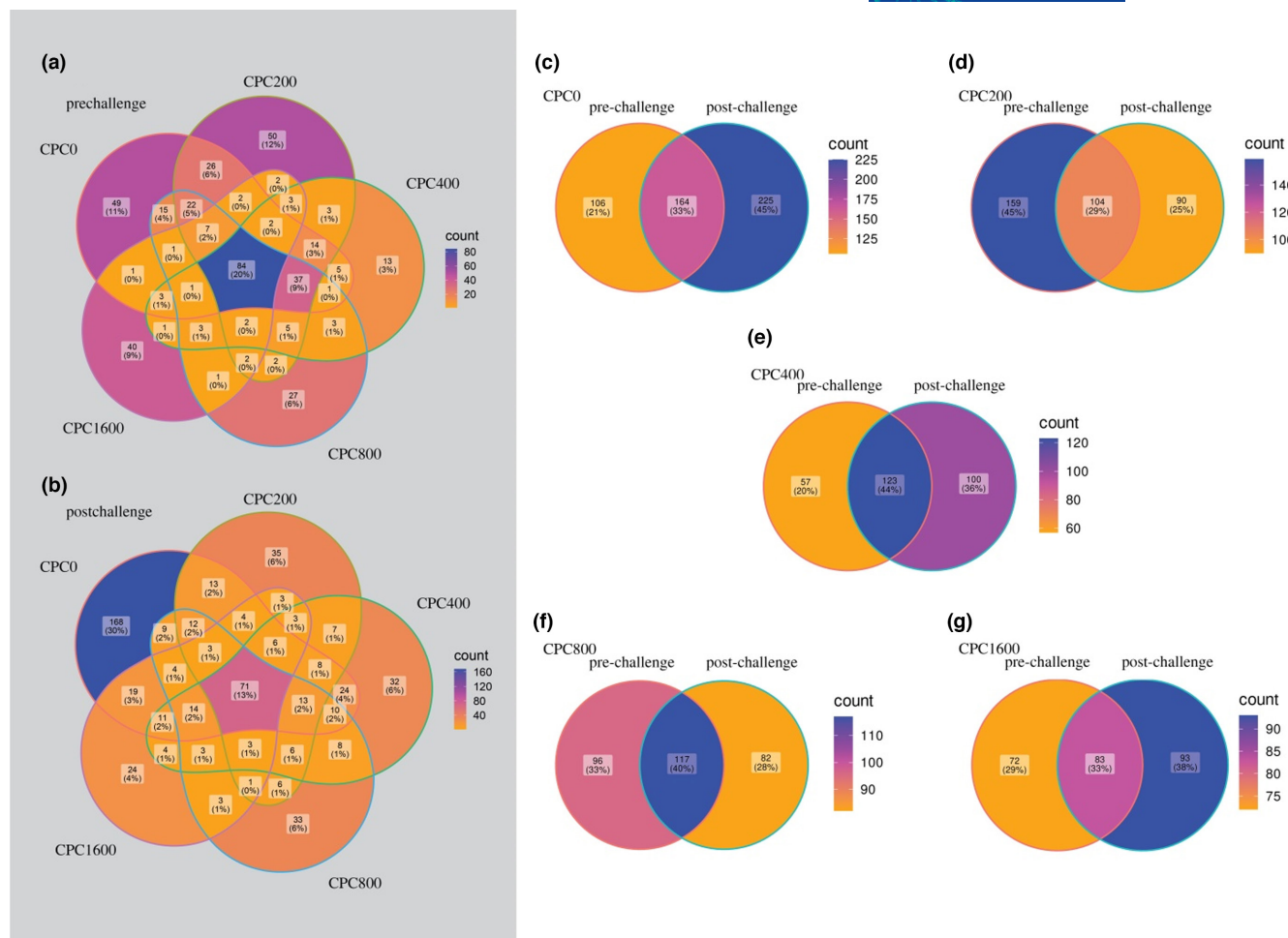


FIGURE 5 (a–g) Venn diagram showing unique and shared operational taxonomic units (OTUs) between pre-challenged and post-challenged *Penaeus vannamei* fed with different levels of C-Phycocyanin (CPC).

CPC₀ (Figure 5c) and CPC₁₆₀₀ (Figure 5g) groups, whereas a greater number of bacterial genera are shared by pre-challenge and post-challenge groups in CPC₄₀₀ (Figure 5e) and CPC₈₀₀ (Figure 5f).

3.7.2 | Changes in gut microbial community composition

The top 10 most abundant bacterial phyla (Figure 6a) and genera (Figure 6b) throughout the gut between the pre-challenge and post-challenge groups are shown in Figure 8. The most highly represented phyla in the gut of all the pre-challenged (52.2%–70.4%) and post-challenge (61.5%–81.3%) groups were observed to be Proteobacteria. Other divisions consistently found in both the groups were Actinobacteria and Bacteroidetes. In all the pre-challenge samples, the presence of Planctomycetes was dominant; however, the phyla were found to be reduced in the post-challenge groups.

The 10 most abundant bacterial genera were also compared and found that the guts of all the pre-challenged groups were dominant in *Ruegeria* sp. except that of CPC₂₀₀, in which *Halocynthiaibacter* sp.

(13.4%) is dominant. However, the gut of CPC₀ harboured large community of *Ruegeria* sp. (35.9%) than other groups. The gut of all the post-challenge groups but CPC₀ was dominant in *Halocynthiaibacter* sp. followed by Rhodobacteriaceae.

3.7.3 | Microbial diversity

The α -diversity of gut microbiome throughout the pre- and post-challenge treatments was examined based on Shannon's (H) diversity, Chao1 richness and Pielou's evenness indices (Figure 7). A comparison of the α -diversity of microbes between the gut of pre- and post-challenge groups revealed that the diversity and evenness reduced post challenge in all the treatments except that of the control (CPC₀). A reduction in microbial species richness was observed throughout the gut of treatment groups (CPC₂₀₀, CPC₄₀₀, CPC₈₀₀ and CPC₁₆₀₀) than the control (CPC₀). As expected, PCoA of weighted Unifrac distance matrices revealed differences in bacterial community structure between the pre- and post-challenge groups (Figure 8), and the clustering was observed to be based on the health status and feed of the shrimp.

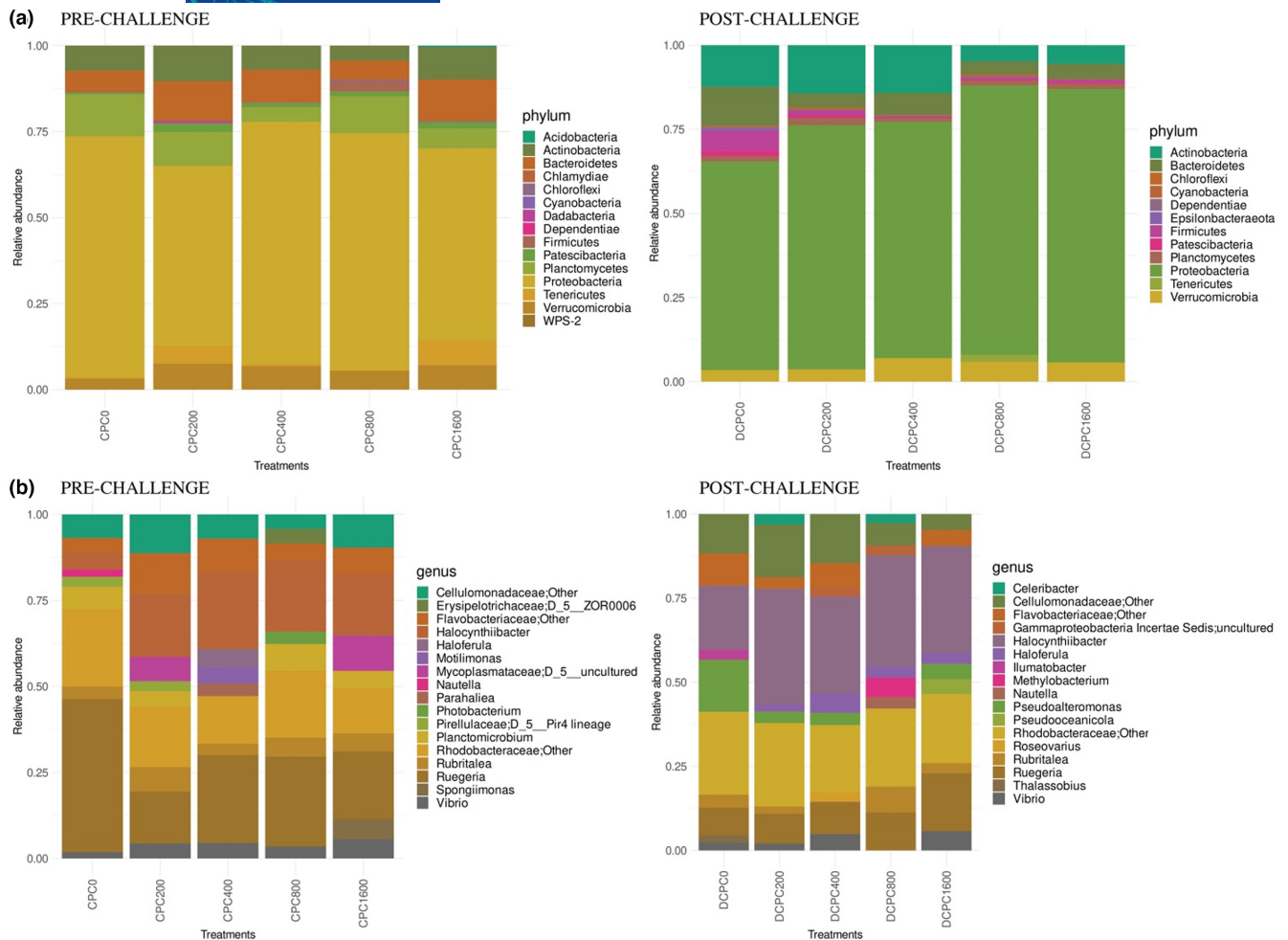


FIGURE 6 Relative abundance of 10 major bacterial phyla (a) and genera (b) in *Penaeus vannamei* fed with different levels of C-Phycocyanin (CPC).

3.8 | Gene expression

To determine the transcriptional response of shrimp to dietary supplementation with CPC, mRNA expression of three immune genes, *ProPO*, *SOD* and *HSP70* were evaluated in *P. vannamei* subjected to bacterial challenge. Dietary CPC significantly up-regulated the expression of branchial *ProPO* mRNA transcript levels compared to control in the pre-challenged shrimps. A significant increase was observed in the group fed with CPC₄₀₀. The *ProPO* transcript levels were higher in the treatment groups compared to the control among the post-challenged shrimps, though the difference was not significant (Figure 9). The relative expression of *SOD* transcripts is shown in Figure 10. The addition of dietary CPC reduced the gene expression level of *SOD* transcript levels in the treatment groups compared to the control with a significant decrease ($p < 0.05$) observed in CPC₈₀₀ in the pre-challenged shrimps. Similarly, the *SOD* transcript level was also significantly lower in the treatment group in the post-challenged shrimps compared to control, except for CPC₂₀₀. Additionally, CPC addition to diets also resulted in a significant increase in CPC₄₀₀ in the post-challenged shrimps. Conversely, the mRNA transcript levels of both *ProPO* and *SOD*

in the post-challenged shrimps were lower compared to those in the pre-challenged shrimps. For *HSP70*, dietary supplementation resulted in no significant changes in the mRNA levels in treatment groups in pre-challenged shrimps (Figure 11). Contrarily, in the post-challenged shrimps, the *HSP70* levels were significantly up-regulated in CPC₁₀₀, CPC₂₀₀ and CPC₄₀₀ but down-regulated in CPC₁₆₀₀ compared to the control.

3.9 | Histopathology

Histomorphology of the HP of *P. vannamei* fed with graded levels of CPC and challenged with *V. campbellii* LB10 is shown in Figure 12a–h. The sections of CPC₀ after challenging (AC) were with evenly distributed B cells and R cells in the HP tubules with very few F cells (Figure 12a). In contrast, in CPC₅₀ (AC) R cells were predominant with condensed nucleus. Infiltration of haemocytosis as well as increased inter-tubular space filled with eosinophilic material was observed (Figure 12b). In CPC₁₀₀ (AC), B and F cells were predominant (Figure 12c) while CPC₂₀₀ (AC) showed B cells and very few F cells (Figure 12d). In CPC₄₀₀ (AC), B cells were predominant (Figure 12e).

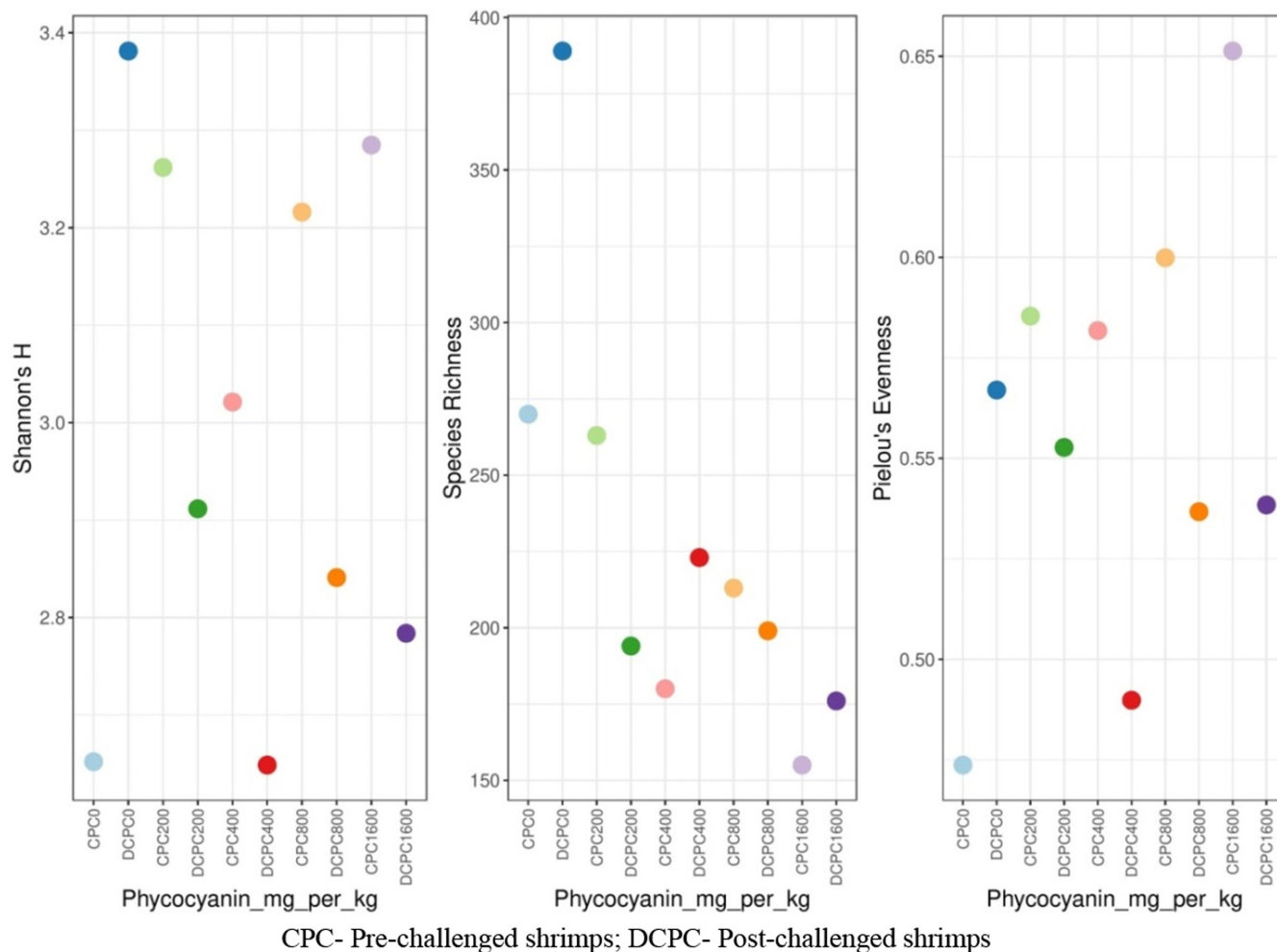


FIGURE 7 Dynamic changes in the α -diversity of gut bacterial communities between pre-challenged and post-challenged *Penaeus vannamei* fed with different levels of C-Phycocyanin (CPC).

with increased inter-tubular space (ITS). In CPC₈₀₀ (AC) and CPC₁₆₀₀ (AC), the presence of B, F and R cells was noticed with regenerative E cells (Figure 12f,g). In CPC₀ without challenging with *V. campbellii*, LB10 showed healthy HP tubules with all types of cells such as B, F, R and E cells (Figure 12h).

4 | DISCUSSIONS

There are reports on the isolation of *Arthrospira* sp. from Indian waters (Raghuraman et al., 2021; Uma et al., 2020) and authors revealed that the fresh isolates of microalgae from a locality have better growth performance and biomass production (Lee et al., 2014; Sandeep et al., 2018). The physicochemical parameters of an ecosystem can influence the abundance and growth kinetics of microalgae to a great extent. Hence, for better growth and production the local strain of microalgae can be used. In the present study, *Arthrospira* sp. has been isolated from the bloom that occurred in an estuarine ecosystem in Southern India. *A. maxima* has thicker sheath appeared to cover the deep ribs as compared to *A. platensis*. Moreover, on solidified media, *A. maxima* displays higher gliding motility than

A. platensis. Hence, the morphological features of isolated species were similar to the characteristics of *A. maxima*. Earlier studies reported the presence of intense blue water-soluble pigment CPC up to 14% of *Arthrospira* sp. biomass (Boussiba & Richmond, 1980). Similarly, in the present study also the content of CPC from *Arthrospira* sp. was about 14% of the biomass. Various properties of CPC such as antioxidant, anti-inflammatory, antifungal, antitumor, immunostimulant, hepatoprotective and neuroprotective have been well documented by different authors (Fernández-Rojas et al., 2014; Romay et al., 2003). Even though the effect of dietary CPC in fish was demonstrated by earlier studies (Biabani Asrami et al., 2019; El-Araby et al., 2022), the application of CPC in the diet of penaeid shrimp has not been studied. Hence in the present study, the dietary effect of CPC in *P. vannamei* has been studied with special reference to growth, survival and immunity.

Our findings showed significant enhancement of growth in *P. vannamei* fed with CPC₈₀₀ and CPC₁₆₀₀ compared to other treatments are in agreement with the earlier studies on guppy, *Poecilia reticulata* (Biabani Asrami et al., 2019) and Nile tilapia *Oreochromis niloticus* (El-Araby et al., 2022) and using CPC. The highest total WG was reported in a dietary supplementation of 1000mg/kg CPC

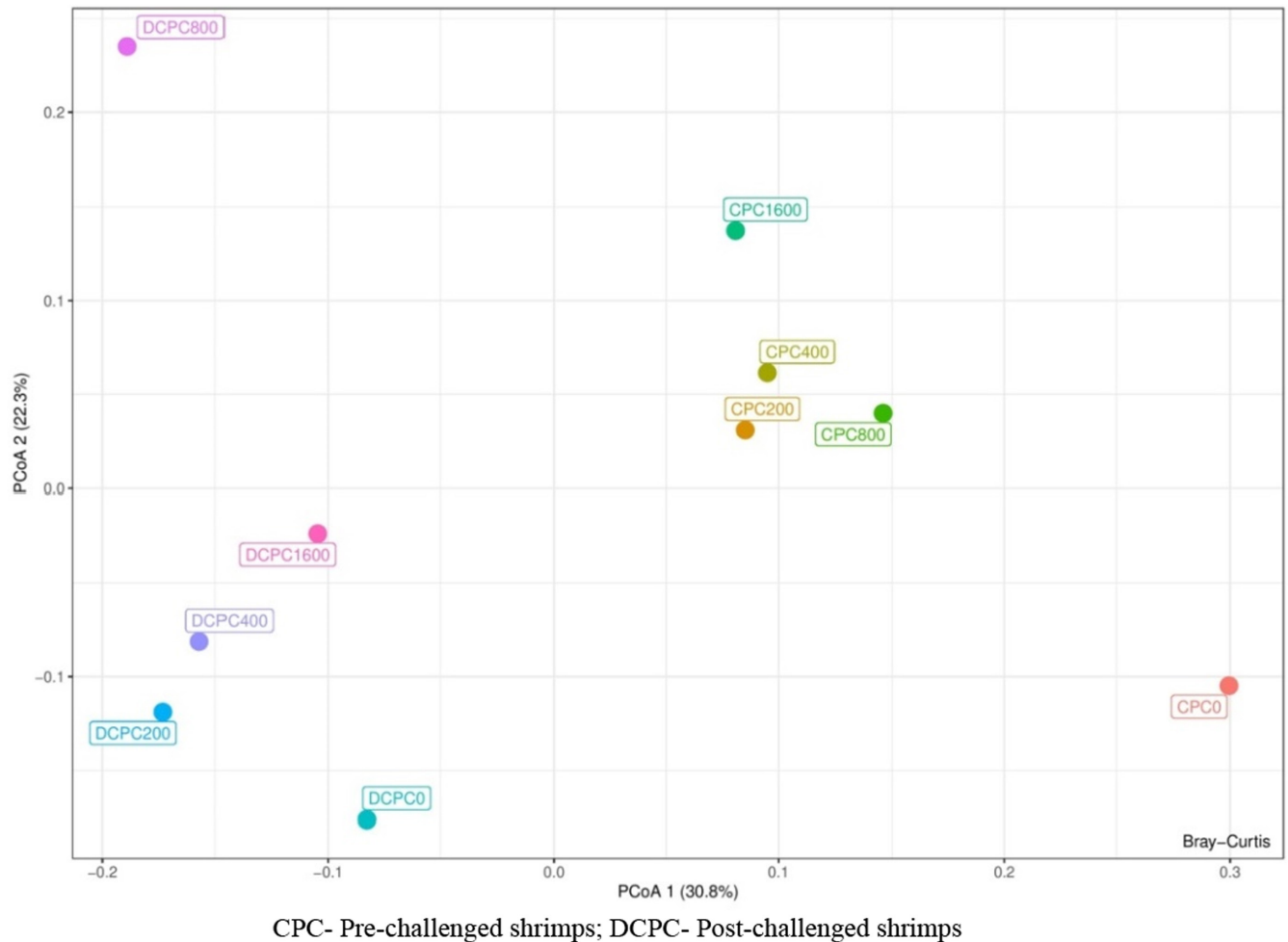


FIGURE 8 Principal coordinate analysis (PCoA) plot between pre- and post-challenged *Penaeus vannamei* fed with graded levels of C-Phycocyanin (CPC).

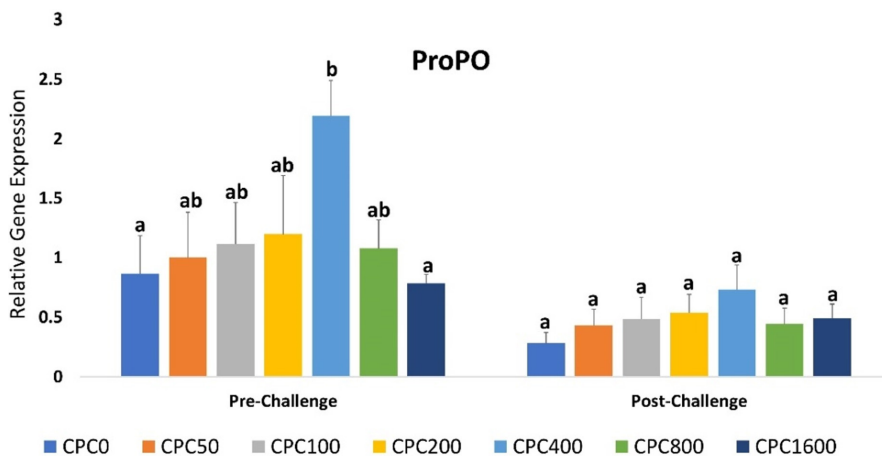


FIGURE 9 The relative expression of ProPO in different treatments.

(El-Araby et al., 2022). The improved growth on supplementation of CPC isolated from *Arthrospira* sp. could be the result of its influence on intestinal flora for the digestion of indigestible constituents of the feed as well as enhanced digestive enzyme activity (Hassaan et al., 2021). Moreover, CPC scavenges reactive oxygen and nitrogen species and prevents oxidative damage in the cells (Fernández-Rojas et al., 2014).

In the present study dietary supplementation of CPC (CPC₄₀₀ to CPC₁₆₀₀) exhibited an increasing trend of THC, SGH and LGH which was in line with the result of Prabu et al. (2018). They have reported increase in haematological parameters in *P. vannamei*, when they were fed with a diet incorporated with leaf powder of *Syzygium cumini*. Haemocytes play crucial role in cellular immune response of shrimps (Ananda Raja et al., 2017). They are important

FIGURE 10 The relative expression of SOD in different treatments.

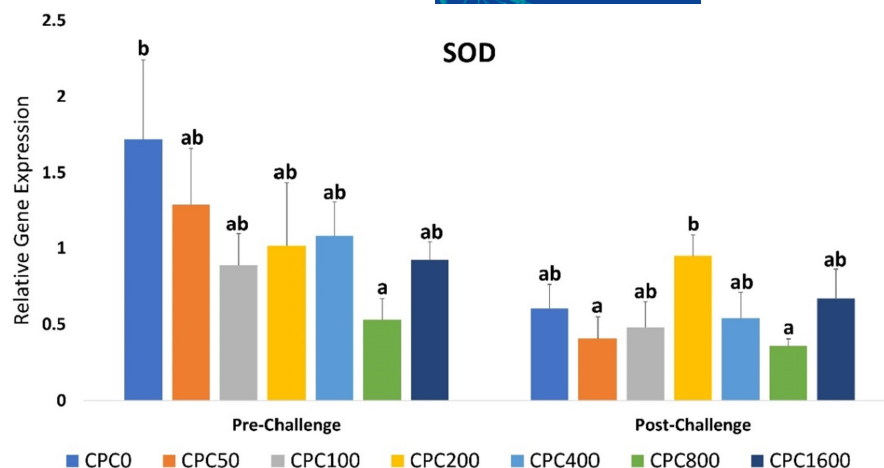
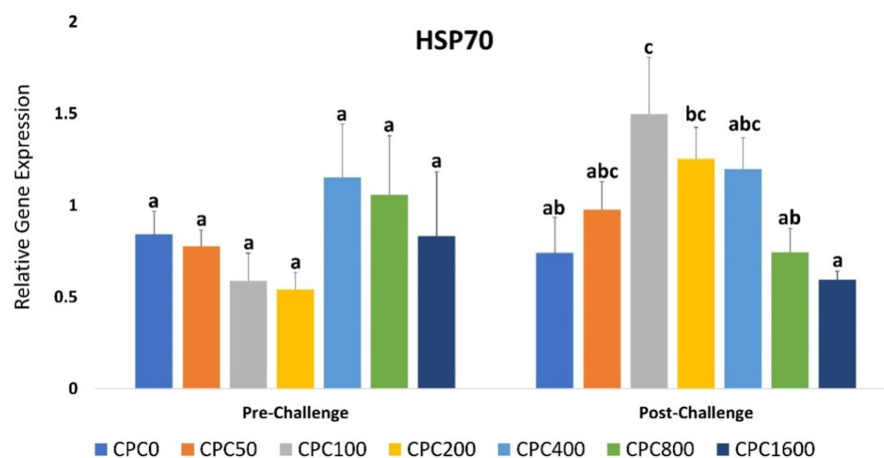


FIGURE 11 The relative expression of HSP70 in different treatments.



for clotting and purging of foreign bodies, hardening of exoskeleton, etc. in crustaceans (Song & Hsieh, 1994). The abundance of haemocytes is due to several reasons like various physiological conditions, reproduction, health and nutritional status of shrimp (Yeh & Chen, 2008).

In the present study, bacterial challenge was carried out using *V. campbellii* LB10 which was found highly virulent in the earlier studies (Kumar et al., 2021). The LD₅₀ of this strain caused 50% mortality among control and CPC₅₀ groups while no mortality was observed among treatments with a higher level of CPC (Figure 4) other than CPC₁₀₀ where 25% mortality was observed. This result suggested the immunomodulatory role of phycocyanin in the shrimp diet.

The relative abundance of dominant phyla, Proteobacteria, is observed in *P. vannamei* infected with *V. parahaemolyticus* (Zhang et al., 2021). Proteobacteria are regarded as the most stable bacteria in the intestine of *P. vannamei* (Li et al., 2018). Proteobacteria are known to participate in carbon utilization and nitrogen transformation in both healthy and diseased shrimps (Duan et al., 2020). The increase in Proteobacteria is also attributed to the risk of diseases (Fan & Li, 2019). Bacteroidetes and Actinobacteria are also reported as the most dominant phyla after Proteobacteria (Li et al., 2018) depending on the environment and diet compositions in healthy and

diseased shrimps. The dominant genus found in the pre-challenge group was observed to be *Ruegeria*, which was isolated mainly from marine habitats and is used as a probiotic. The dominance of *Ruegeria* in the healthy *P. vannamei* gut was also reported by Fan and Li (2019). Some previous studies also reported the relative abundance of Rhodobacteriaceae (Liu et al., 2019). As found in the present study, Rhodobacteriaceae family was reported to be largely involved in carbon/sulphur cycling (Liu et al., 2019). Only two species of *Halocynthiaibacter* sp. namely *H. namhaensis* and *H. arcticus* were described so far (Lee et al., 2016). *H. arcticus* was reported to be positive for alkaline phosphatase, lipase, and alpha-glucosidase and can hydrolyse Tweens 40, 60, and 80 (Lee et al., 2016). Due to the presence of three arsenate reductases, this bacterium can be used in bioremediation of arsenic-contaminated environments. There is a dominance of *Methylobacterium* in DCPC800, which is a potentially important genus that protects the host against pathogens in fish skin. It also produces poly-β-hydroxybutyrate, degrading short-chain fatty acids. These activities inhibit the growth of potential pathogens (Korotkova et al., 2002).

The microbial community in the post-challenge groups clustered together and was separate from those in the pre-challenge groups, showing that the clustering is based on the health status of the shrimp. The current study revealed the change in the composition

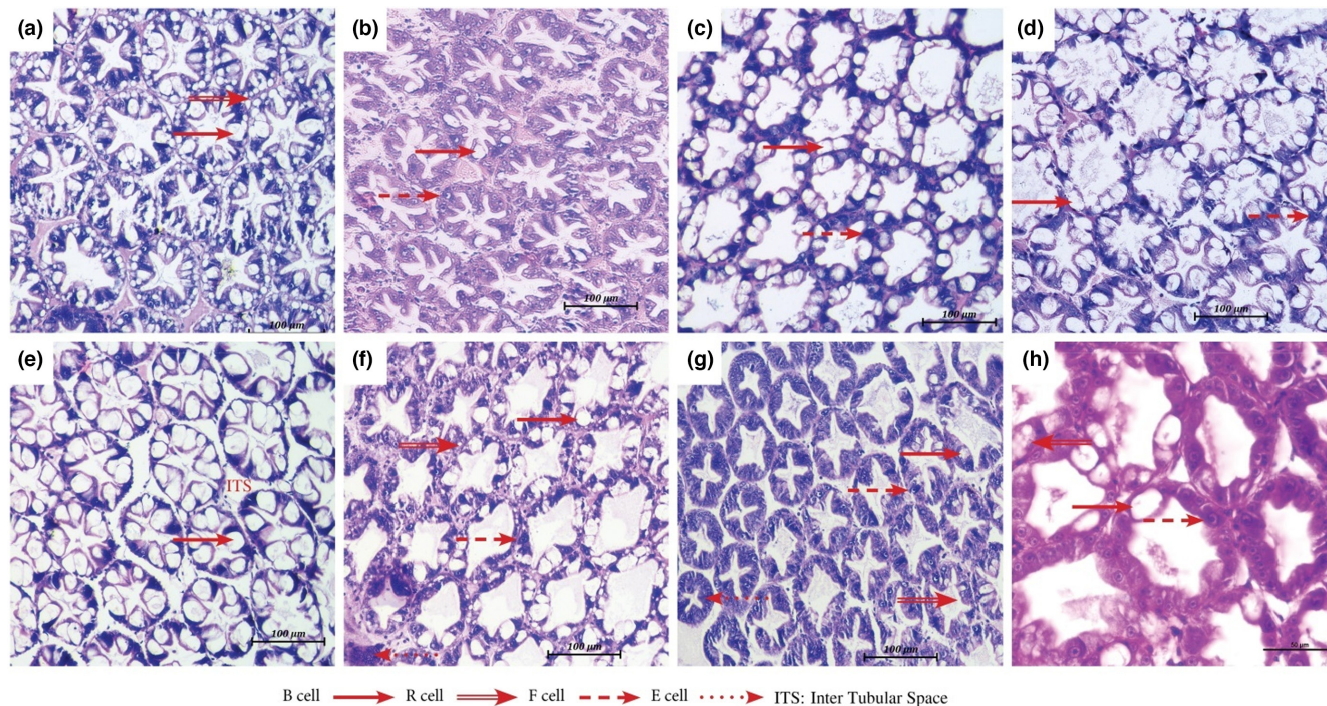


FIGURE 12 Histopathology of sagittal sections of the HP of *Penaeus vannamei* fed with different levels of C-Phycocyanin (CPC) and challenged with *Vibrio campbelli* LB10. (a) CPC₀ after challenging: Noted evenly distributed B and R cells in the hepatopancreatic (HP) tubules with very few F cells. (b) CPC₅₀ after challenging: R cells were predominant with condensed nucleus. Increased inter-tubular space filled with eosinophilic material and infiltration of haemocytes was observed. (c) CPC₁₀₀ after challenging: B and F cells were predominant. (d) CPC₂₀₀ after challenging: HP tubules are predominantly found with B cells and very few F cells. Focal areas of inter-tubular space are filled with eosinophilic material. (e) CPC₄₀₀ after challenging: Abundant B cells with increased inter-tubular space. (f) CPC₈₀₀ after challenging: B cells were predominant while F and R cells were equally distributed. Haemocyte infiltrations in the inter-tubular space were noticed. Regenerative E cells were also evident. (g) CPC₁₆₀₀ and after challenging: F cells were predominant with less number of B cells and scanty R cells. Regenerative E cells were evident in the proximal region. (h) CPC₀ without challenging: Noted healthy HP tubules with all types of cells such as B, F, R and E cells.

of intestinal microbiota in the gut of *P. vannamei* concerning dietary incorporation of CPC before and after the *V. campbelli* LB10 challenge. There was a shift in the relative abundance of dominant phyla and genera along with the bacterial diversity, evenness and richness. Therefore, dietary incorporation of CPC followed by *Vibrio* infection alters the gut microbiome in *P. vannamei*.

Previous studies of vibriosis in shrimp were related to immune response and pathogenesis, and little focus was given to the response of functional nutrients and gut microbiome. Alterations in the gut microbiome are associated with the severity and stage of the disease (Zhang et al., 2021). Results showed that the infection of *V. campbelli* reduced the bacterial diversity and evenness in the guts of *P. vannamei*. The reduction in the richness of bacterial communities was observed in all the treatment groups than in the control.

The shrimp's immune responses include both humoral and cellular factors. Phagocytosis and respiratory burst (production of ROS) are activated in shrimp's innate immunity mechanisms against infection. The *ProPO* system plays a key role in the non-specific immune system in invertebrates (Ananda Raja et al., 2017). Results from our study indicated increased *ProPO* gene expression with dietary supplementation of CPC, and the highest gene expression

was observed in groups fed with 400mg of CPC per kg diet in pre-challenged shrimps indicating the possible role of CPC to activate the phenoloxidase system in *P. vannamei*. The dietary treatment groups in the post-challenged shrimps did not exhibit any deviation in *ProPO* transcript levels. SOD, a systolic enzyme that converts the excess of superoxide anion (O_2^-) to less harmful hydrogen peroxide (H_2O_2), constitutes the first line of antioxidant defence systems against superoxide radicals (ROS) (Wang et al., 2007). In the present study, SOD gene levels in the CPC fed shrimps were not different from control except for CPC₈₀₀ in pre-and post-challenged shrimps and CPC₂₀₀ in post-challenged shrimps, which might be due to decreased oxidative stress and free radical activities for the maintenance of superoxide anion levels during the feeding trial. In contrast, the inclusion of astaxanthin (Wang et al., 2015) or fulvic acid (FA) significantly improved SOD activity in the Chinese shrimp *P. chinensis* (Zhao et al., 2001) and modulated the immune response in *P. vannamei* (Fierro-Coronado et al., 2018). The decrease in SOD mRNA levels observed in CPC₈₀₀ could indicate the decreasing superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) levels, indicating a decline in ROS production. The increased SOD transcript levels observed in CPC₂₀₀ in post-challenged shrimps suggests that dietary inclusion of CPC and bacterial challenge probably resulted in ROS accumulation.

The anti-stress protein, heat shock proteins (HSPs), such as *HSP70*, acts as molecular chaperones in the defence mechanisms to mediate humoral and cellular innate immune responses during heat stress, oxidative stress, malnutrition and microbial infections in penaeid shrimps and has been extensively studied in aquaculture (Hickey & Singer, 2004; Multhoff, 2007). Results of the expression of the *HSP70* gene showed a similar trend to the *SOD* gene expression by increasing its expression in the post-challenged shrimps fed with CPC, while dietary supplementation of CPC did not induce any significant changes in the *HSP70* gene expression in the pre-challenged shrimps. Feeding *Macrobrachium rosenbergii* with immunostimulants (*Saccharomyces cerevisiae* and β -glucan) significantly increased the gene expression of immune-related genes including *ProPO*, *HSP70* and *SOD* (Han et al., 2021). Previous studies have indicated that *A. platensis* stimulated the innate immunity of *P. vannamei* (Lin et al., 2010). A combination of live bacteria *Lactobacillus pentosus* and *A. platensis* exerted synergistic effects for improving growth and immune response in *P. vannamei* (Liu et al., 2022). The gene modulation in *P. vannamei* in response to the diets observed in the present study indicates the influence of CPC on the immune response which needs to be confirmed by further studies.

The B cells or blister-like or Blastozellen are responsible for the production of digestive enzymes in HP, nutrient accumulation as well as transport of digested materials (Ananda Raja et al., 2017). E cells or embryonic cells play an important role in tubular epithelium renewal (Ambasankar et al., 2022; Sonakowska-Czajka et al., 2021). Healthy HP of *P. vannamei* show intact B, R, E cells, tubular lumen and rounded epithelial tubule (Ananda Raja et al., 2017). In the present study, the presence of B, F, R and regenerative E cells in CPC₈₀₀ and CPC₁₆₀₀ even after challenging with *V. campbellii* LB10 shows the health status of the HP and supports the immunomodulatory effects of CPC in *P. vannamei*.

5 | CONCLUSION

Utilization of nutraceuticals in the diet of shrimps to reduce disease-related issues in the shrimp farming is the need of the hour. Dietary supplementation of CPC at 800mg/kg feed significantly enhances the growth performance of *P. vannamei* reared in tank systems. In addition, the dietary supplementation of CPC improves the health status of *P. vannamei* in terms of haematology, gut microbiome, histopathology and relative gene expression. The results of the study will help in the development of functional feed for penaeid shrimps to prevent bacterial diseases in farming conditions.

AUTHOR CONTRIBUTIONS

Sandeep K. P.: Conceptualization and planning of work, conduct of experiments, analysis and interpretation of data, manuscript writing. **Jesudhas Raymond Jani Angel:** Analysis of gut microbiome, interpretation of data, manuscript editing. **Sivaramakrishnan Thirugnanamurthy:** Formulation of experimental diets, analysis and interpretation of data and manuscript editing. **Sudhin S.:**

Conduct of the experiments, water quality analysis and analysis of growth parameters. **Suganya N.:** Analysis of gut microbiome. **Ramalingam Ananda Raja:** Analysis and interpretation of haematology and histopathology. **Sujeet Kumar:** Analysis and interpretation of gut microbiology. **Sherly Tomy:** Analysis and interpretation of gene expression. **Jagabathula Syama Dayal:** Interpretation of data on feeding trials, manuscript editing. **Balasubramanian Changaramkumarth Paran:** Interpretation of data, manuscript editing. **Paramita Banerjee Sawant:** Interpretation of data, manuscript editing. **Shekhar Mudagandur Shashi:** Conceptualization and planning of work, Interpretation of data, manuscript editing. **Chadha Narinder Kumar:** Conceptualization and planning of work, interpretation of data, manuscript editing. **Ambasankar Kondusamy:** Conceptualization and planning of work, mobilization of funds, interpretation of data, manuscript editing.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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