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EDITED BY
Kumbukani Mzengereza,
Mzuzu University, Malawi

REVIEWED BY
Zhenlu Wang,
Guizhou University, China
Amit Ranjan,
Tamil Nadu Fisheries University, India

*CORRESPONDENCE
Mohamed Ashour
microalgae_egypt@yahoo.com
Abdallah Tageldein Mansour
amansour@kfu.edu.sa
Ehab Mohamed
ehab.reda@uaeu.ac.ae

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Seaweed (*Pterocladia capillacea*) nanoparticles improves growth performances, digestive enzymes, antioxidant activities, innate immunity, and related-immunity gene expressions of *Litopenaeus vannamei*

Mohamed Ashour[1*], Mohamed M. Mabrouk[2], Ahmed I. A. Mansour[1], Mohammed A. E. Naiel[3], Abdallah Tageldein Mansour[4*], Ehab Mohamed[5*] and Ahmed F. Abdelhamid[2]

[1]Aquaculture Division, National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt, [2]Fish Production Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, [3]Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig, Egypt, [4]Animal and Fish Production Department, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa, Saudi Arabia, [5]Department of Integrative Agriculture, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Abu Dhabi, United Arab Emirates

This work evaluated the effects of dietary supplementation with the seaweed, *Pterocladia capillacea*, nanoparticles (SN) on the growth, whole-body composition, digestive enzyme activities, feed efficiency, immunological response, antioxidant activity, and gene expression of the whiteleg shrimp, *Litopenaeus vannamei*. The SN form was conducted using a Planetary Ball Mill PM 400. The particle size of the SN was verified through Dynamic Light Scattering (DLS) analysis. The DLS showed that the mean particle sizes of SN were between 151 nm (13.6%) and 835 nm (64%). Throughout 60 day experimental trial, postlarvae (PLs) of *L. vannamei* were subjected to one of the following five feeding groups. The first group is a commercially available shrimp feed as a basal diet without any seaweed supplementation, functioning as a negative control ($C_{0\%}$). A second group received the commercial feed supplemented with 2% (20 g/kg) dried seaweed powder ($SP_{2\%}$), functioning as a positive control. The remaining three shrimp groups were fed diets supplemented with seaweed nanoparticles (SN) at concentrations of 0.5% ($SN_{0.5\%}$), 1% ($SN_{1\%}$), and 2% ($SN_{2\%}$), respectively. 750 postlarval (0.053 g/PL) were allocated to five experimental diet groups. Each group consisted of 150 PLs (triplicate). The PLs were fed their corresponding regime three times a day at 10% of their body weight. The results revealed that, compared with those of the positive ($C_{0\%}$) or negative ($SP_{2\%}$) controls, with the increasing dietary supplementation levels of SN, especially $SN_{2\%}$, the growth (FW, WG, and SGR), digestive enzymes (amylase and lipase activities), carcass composition (protein and lipid contents), nutrient efficiency (FI, PI, FER, FCR, and PER), antioxidant activities (SOD and CAT), innate immunity activities (LYS and MDA), and related-

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immunity gene expressions (*p53*) of *L. vannamei* were significantly improved. In conclusion, these findings concluded that applying nanotechnology tools enhances feed additives and significantly maximizes the positive effects of these additives on *L. vannamei* growth, health, and overall production. Further research is required to understand and explain how seaweed nanoparticles affect these shrimp's physiological state and upgrade some immune-related gene expressions.

KEYWORDS

amylase, FcR, lipase, macroalgae, nanotechnology tools, seaweed, shrimp dietary supplementation, whiteleg shrimp

1 Introduction

The aquaculture industry now seems considerably increasing due to the human population's expansion and increased emphasis on healthy nutrition (Abbas et al., 2023). Farmed seafood is now a vital key diets proposed to boost human wellness (Kwasek et al., 2020). Nowadays, marine shrimp farming has a dramatic rise and is considered a key element in feeding developing nations (Mansour et al., 2022a). Even so, several problems still face aquaculture's progress. These obstacles include a rise diet prices, diet formulation, low diet efficiency, low water quality efficiency, low survivability, lack of supply of diet ingredients, climate change, harmful environmental impact, farmer practices, increases in water pollution, and maintaining good water quality (Magouz et al., 2021b, a; N'Souvi et al., 2024; Widiasa et al., 2023; Suzuki and Nam, 2023). Among all these obstacles, formulating better feeds with high diet efficiency promises to positively influence shrimp's development, robustness, and efficient production (Mansour et al., 2022c; Ashour et al., 2024b, a; Ahmed et al., 2019).

Shrimp consumption has risen significantly all over the world. Among all farmed shrimp species worldwide, more than 70% are represented by *Litopenaeus vannamei* species (Pedrazzani et al., 2023; Chen et al., 2024). To meet the rising global demand for shrimp, several strategies have been developed to improve shrimp diets and nutrition (Emerenciano et al., 2022; Khanjani et al., 2022). The inclusion of natural feed additives into shrimp diets is a one of the most effective and widely strategy can improve and develop the shrimp aquaculture production (Marimuthu et al., 2022). Diet feed additives play a key role in enhancing shrimp's growth, immunity, antioxidant and gene expression (Ceseña et al., 2021; Mustafa and Al-Faragi, 2021; Ashour et al., 2024a).

Algae (microalgae and seaweed) are extensively utilized in several industries. These applications include algae used as aquafeed additives (Abbas et al., 2023), dietary supplements for human nutrition (Hamid et al., 2025), plant growth enhancers (Mansour et al., 2022b), pharmaceutical ingredients (Leung, 2025), antimicrobial agents (Osman et al., 2010, 2020), bioenergy sources (Elshobary et al., 2021), components in cosmetic products (Kaur and Khattar, 2025), and sustainable tools for phytoremediation (Al-Saeedi et al., 2023).

Among the different families of seaweed, red seaweeds family (Rhodophyceae) stand out due to their rich composition of bioactive compounds. These include polysaccharides, vitamins, alkaloids, flavonoids, phenolic compounds, fatty acid methyl esters, hydrocarbons (Sanjeewa et al., 2018). These biomaterials showed protective effects on aquatic organisms by offering a range of beneficial activities including growth stimulation benefits, anti-inflammatory effects, immune system enhancement, antimicrobial effects, and antioxidant properties (Yazici et al., 2024). In a pioneer study conducted by Zhang et al. (2023), the influence of nine different macroalgae as aquafeed additives on various physiological indicators of *L. vannamei* over a 28-day feeding trial has been investigated. These macroalgae belonged to three microalgae groups: brown macroalgae (one speices: *Sargassum ilicifolium*), red seaweeds (three species: *Betaphycus gelatinae Acanthophora spicifera*, and *Gracilaria bailiniae*), and green seaweeds (five species: *Caulerpa linum, Caulerpa sertularioides Caulerpa lentillifera, Ulva lactuca*, and *Caulerpa racemosa*). Among all the tested species, the red macroalgae *A. spicifera* showed the most significant enhancement in immune responses, antioxidant potential, and gene expression linked to antioxidation in *L. vannamei* (Zhang et al., 2023). However, the efficacy of algae feed additives for shrimp and fish is significantly influenced by several factors like the concentration and the form of addition in which they are administered. Thus, it generates recognition that adding appropriate concentration and the form of addition would improve growth rates and feed efficiency while also increasing aquatic animals' immune systems and significantly boosting their resistance to disease (Sharawy et al., 2022).

In the nanotechnology process, materials are manipulated at the nanoscale, usually ranging from 1 to 100 nanometers (Ashour et al., 2023). By reducing bigger chemical compounds to tiny particles, nanotechnology methodology gives these biomaterials new physicochemical characteristics (Mansour et al., 2022a). Currently,

in aquaculture, there's growing interest in supplementing diets with nanoparticles of macroalgae over traditional additives (powder-particle form, liquid-extract form, or powder-extract form). Although nanotechnology is recently involved in various branches within aquaculture, the use of macroalgae-derived nanoparticles as a supplement in aquaculture feeds remains an underexplored area.

Among red seaweeds species, *Pterocladiella capillacea* is recognized for its rich content of polysaccharides and a variety of bioactive compounds (Ismail et al., 2023), including phenolics, fatty acids, flavonoids, vitamins, and alkaloids. These constituents contribute to a wide range of beneficial biological activities, such as antioxidant, antimicrobial, anti-inflammatory, immune-enhancing, and growth-promoting effects, which can help protect aquatic organisms from various harmful conditions (Kannan et al., 2024).

This study investigates how supplementing the diet of whiteleg shrimp *L. vannamei* postlarvae with red macroalgae (*Pterocladiella capillacea*) nanoparticles influences the shrimp's growth, innate immunity, antioxidant activities, digestive enzyme activities, nutrient efficiency, and related-immunity gene expressions.

2 Materials and methods

2.1 Seaweeds

2.1.1 Preparation

The Rhodophyceae species, *Pterocladiella capillacea*, was harvested from the Alexandria coast of Egypt. After collection, the samples were cleaned and air-dried. The dried samples were fine powder grounded and then stored in dark plastic bags at room temperature for further application (Abbas et al., 2023). Subsequently, the seaweed powder form (SP) was prepared to synthesize seaweed nanoparticles (SN) form. This process was conducted at the Egyptian Petroleum Research Institute (EPRI) using a Planetary Ball Mill PM 400, equipped with four grinding stations. The particle size of the SN was verified through Dynamic Light Scattering (DLS) analysis using a Zetasizer Nano Series HT, Nano-25, and Malvern instruments. The DLS showed that the mean particle sizes of SN were between 151 nm (13.6%) and 835 nm (64%) (Supplementary Figure S1A).

2.1.2 Characterization

Based on the work conducted by Ashour et al. (2020), the carcass whole-body composition of SP of carbohydrate, protein, lipid, and ash was reported as presented in Supplementary Table S1. Moreover, SP showed significant content of fatty acids (polyunsaturated fatty acids, monounsaturated fatty acids, and saturated fatty acids) and amino acids (essential and nonessential amino acids). Furthermore, the SP water extract showed a significant amount of phenolic content, carotene content, total antioxidant, flavonoid content, and, as presented in Supplementary Table S1 (Ashour et al., 2020). Based on our previous study, several characterizations of SN were performed as reported by Mansour et al. (2022b). The Scanning Electron Microscopy (SEM) analysis reflected the morphological aspects of SN (Supplementary Figure S1B). The Fourier transform infrared spectroscopy (FTIR) analysis identified several types and numbers of functional groups on the surface of SP, like biomolecules, proteins, polysaccharides, and aromatic compounds, which have been identified to contain functional groups such as hydroxyl, amino, aldehydes, ketones, carboxyl, carboxylates, carbonyl, phosphoryl, esters, and sulfide (Supplementary Figure S1C).

To further validate the bioactive compound and give more insights into the composition of the SN, spectroscopic analysis was conducted on the SN crude extract of SN using ultraviolet (UV) spectra analysis. The UV-visible analysis of SN confirmed that the SN contains several glycosides, alkaloids, pigments, and flavonoid compounds when scanned spectroscopically in the wavelength ranging from 200 nm to 800 nm (Supplementary Figure S1D). Moreover, the Brunauer Emmett Teller (BET) analysis demonstrated accurate measurement of the specific surface area of SN (128 m^2/g), besides other related measurements as presented in Supplementary Table S2 and Supplementary Figure S2.

2.2 Experimental diets preparation

Throughout 60 day experimental trial, postlarvae (PLs) of *L. vannamei* were subjected to one of the following five feeding groups. The first group is a commercially available shrimp feed (Aller-Aqua, Egypt; the composition is detailed in Table 1) as a basal diet without any seaweed supplementation, functioning as a negative control ($C_{0\%}$). A second group received the commercial

TABLE 1 Biochemical compositions of the experimental diets.

Chemical composition (%,dry matter basis)	Experimental Groups*				
	$C_{0\%}$	$SP_{2\%}$	$SN_{0.5\%}$	$SN_{1\%}$	$SN_{2\%}$
Crude protein	40.45	40.60	39.83	40.45	40.40
Crude lipid	7.86	7.85	7.88	7.85	7.89
Crude fiber	3.59	3.68	3.64	3.65	3.60
Ash	9.27	9.38	9.28	9.33	9.29
Gross energy (MJ/kg)	19.90	19.97	19.97	20.19	20.21

*$C_{0\%}$, Control Basel diet; as a negative control; $SP_{2\%}$, Diet contains 2% (20 g/kg diet) powder of seaweed, as a positive control. $SN_{0.5\%}$, $SN_{1\%}$; and $SN_{2\%}$, Diets contain 0.5%; 1%, and 2% of seaweed nanoparticles (5, 10, and 20 g/kg diet, respectively).

feed supplemented with 2% (20 g/kg) dried seaweed powder ($SP_{2\%}$), functioning as a positive control. The remaining three shrimp groups were fed diets supplemented with seaweed nanoparticles (SN) at concentrations of 0.5% ($SN_{0.5\%}$), 1% ($SN_{1\%}$), and 2% ($SN_{2\%}$), respectively.

The addition of nanoparticle additives into the feed diet was adapted from the procedure reported by (Mabrouk et al., 2022). Briefly, the diet was initially ground into a fine powder, and the designated quantities, either seaweed powder (SP) or seaweed nanoparticles (SN), were carefully incorporated separately into the targeted powdered diets, ensuring that it was completely uniform. The appropriate amount of SP and SN was dissolved in a volume of distilled water (DW) and then sprayed equally onto the surface of the corresponding feed batch. The negative control ($C_{0\%}$) diet had an equivalent spray of DW without any seaweed supplement. Sunflower oil (5 mL/kg diet) was sprayed onto all diets after drying (48 hours/40°C) to achieve a final moisture level of approximately 10%. Finally, the prepared feed pellets were then stored (4°C) until use.

2.3 Experimental procedures

Following a 15-day acclimation period, 750 PLs (0.053 ± 0.002 g per PL), were allocated to five groups. Each group consisted of 150 PLs (triplicate). Along the experiment, each group was housed within a concrete pond with a dimension of 4 m × 2 m × 1 m and subdivided into three net enclosures (hapas), with a dimension of 0.7 m × 0.7 m × 1 m, each containing 50 PLs. Over the subsequent 60 days, the PLs were cultivated while maintaining optimal water quality conditions of salinity, pH, ammonia (NH_3), temperature, nitrite (NO_2), and nitrate (NO_3) levels. Salinity, temperature, and pH were measured daily around midday. Ammonia, nitrite, and nitrate concentrations were weekly assessed. These water quality parameters were assessed following the APHA (2005) standard procedures. In all experimental groups, around 10% of the total water volume was exchanged daily with fresh and clean water. During this study, the measured values of water quality indices indicated that temperature (26.0 ± 1.5°C), salinity (27.5 ± 2 ppt), pH (7.6 ± 0.3), ammonia (0.08 ± 0.04 mg/L), nitrate (0.16 ± 0.04 mg/L), and nitrite (0.08 ± 0.02 mg/L) remained within the acceptable ranges for shrimp farming, as reported by Venkateswarlu et al. (2019)_ENREF_5.

2.4 Shrimp

2.4.1 Procedures

The *L. vannamei* PLs_{15} were transported from a private shrimp hatchery located in Kafr El-Sheikh city, Egypt, to the Fish Research Station located in Baltim, Alexandria Branch of NIOF, Egypt. The PLs_{15} were housed in concrete ponds measuring 1 m × 5 m × 5 m, for a 15-day acclimation period to adjust to the experimental culture conditions. The water parameters during the acclimation period included dissolved oxygen levels above 5 mg/L, a temperature of 27 ± 1°C, and salinity of 26.5 ± 1.0 ppt. To maintain water quality, approximately 10% of the total water volume in the concrete culture pond was replaced daily with fresh and clean water. During acclimatization, the shrimp were fed a control basal diet containing 40.45% protein four times daily until full satiation.

2.4.2 Growth and feed efficiency indicators

To evaluate the effectiveness of the feeding regimen on shrimp survival rate, growth performance, and feed efficiency indicators, the following formulas Equations 1–6 were applied:

$$\text{Weight Gain (WG, g)} = \text{Final body weight (g)} - \text{Initial body weight (g)} \quad (1)$$

$$\text{Survival Rate (SR, \%)} = \frac{\text{The total final survived number of shrimp}}{\text{The initial number of shrimp}} \times 100 \quad (2)$$

$$\text{Specific Growth Rate (SGR \%/day)} = \frac{\text{Ln Final body weight} - \text{Ln Initial body weight}}{t} \times 100 \quad (3)$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total consumed feed}}{\text{WG}} \quad (4)$$

$$\text{Feed Efficiency Ratio (FER)} = \frac{\text{WG (g)}}{\text{Feed intake (g)}} \quad (5)$$

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{WG (g)}}{\text{Protein intake (g)}} \quad (6)$$

2.4.3 Biochemical analysis

For biochemical analysis, at the end of the trial, 15 shrimp were randomly sampled from each group (5 individuals/replicate). The selected shrimp were euthanized, homogenized, dried, ground into powder, and stored at −20°C for later application. The dry matter, crude protein, crude lipid, and ash content of the samples were analyzed according to the AOAC (2003) protocol.

2.4.4 Digestive enzyme activities

Following a 24-hour fasting after the experiment finished, 15 individuals were randomly selected from each group (five individuals per replicate) to analyze the activities of digestive enzymes, including amylase and lipase. These enzymes were measured in homogenized digestive gland tissue using colorimetric assay kits following the manufacturer's protocol. Shrimp individuals were euthanized by cryoanesthesia, as described by (Becker et al., 2024). Shrimp muscle tissue and internal organs were carefully dissected, weighed, and homogenized in phosphate buffer solution (PBS) with a pH of 7.4. The homogenized samples were then centrifuged for 20 minutes at 3500 rpm, then the supernatants were collected and

stored at -20°C until analysis. Enzyme activity was assessed using specific kits for lipase and amylase provided by Biodiagnostic Company, Egypt (catalog Numbers: 281001 and AY1050 respectively). Following the protocols of Moss et al. (1999) and Caraway (1959), the absorbance for lipase activity was determined at 580 nm, while amylase was measured at wavelengths of 660 nm.

2.4.5 Antioxidant enzymes activities

Following a 24-hour fasting after the experiment finished, 15 individuals were randomly selected from each group (five individuals per replicate) to analyze the antioxidants enzymes activities. Shrimp whole body samples were dissected, weighed, homogenized, after adding PBS (pH 7.4), centrifuge (20 min, 2,000–3,000 rpm), and the supernatant was carefully collected and stored at – 20 °C until use. The antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and lipid peroxidation (malondialdehyde, MDA) were quantified using the spectrophotometry colorimetric method. Specifically, the CAT, SOD, and MDA activities were assessed using commercial kits obtained from Biodiagnostic Company, Egypt. Following the method described previously by (Nishikimi et al., 1972), the CAT activity (Cat Nos. CA2517) was determined at wavelengths of 560 nm. SOD activity (Cat Nos. SD2521) was determined at wavelengths of 510 nm according to (Aebi, 1984). MDA activity (Cat No. 2529) was determined at wavelengths of 534 nm following the protocol described by Ohkawa et al. (1979).

2.4.6 Innate immunity activities

Following a 24-hour fasting after the experiment finished, 15 individuals were randomly selected from each group (five individuals per replicate) to evaluate lysozyme (LYS) activity. Shrimp whole body samples were dissected, weighed, homogenized, after adding PBS (pH 7.4), centrifuge (20 min, 2,000–3,000 rpm), and the supernatant was carefully collected. LYS activity was quantified using an enzyme-linked immunosorbent assay (ELISA) kit (Cat No.: SL0050FI; SunLong Biotech Co., Ltd., China). The assay principle relies on the enzymatic degradation of *Micrococcus lysodeikticus* cell walls by lysozyme. The LYS activity was measured spectrophotometrically as a decrease in absorbance at 450 nm, according to the manufacturer's protocol conducted by (Harshbarger et al., 1992).

2.4.7 Immunity-related gene expressions

When the trial ended, for each treatment group, three separate shrimp samples (5 whole-body shrimp/replicate) were applied to prepare the RNA analysis. Initially, samples were subjected to two washes using a phosphate-buffered saline solution (PBS; composition of 1.46 mM KH_2PO_4, 2.7 mM KCl, 8 mM Na_2HPO_4, and 137 mM NaCl, pH 7.4). Subsequently, the samples were stabilized for RNA integrity by immersion in RNA later® reagent (Sigma-Aldrich®) at a 1:5 volume ratio and stored at a temperature of -20°C Goncalves et al. (2014). The extraction of total RNA and the subsequent quantitative real-time polymerase chain reaction (qRT-PCR) analysis were conducted following the protocols published by Aguilera-Rivera et al. (2019). Subsequently, for immunity-related gene expressions, the gene-specific primers were detailed in Supplementary Table S3. Using β-actin as a housekeeping gene (Wang et al., 2007), four immunity-related genes of peroxiredoxin, *Prx* (Liu et al., 2020), prophenoloxidase, *PPO1* (Wang et al., 2007), p53-like protein isoform delta, *p53* (Nuñez-Hernandez et al., 2021), and hemocyanin subunit L5, *L5H* (Wang et al., 2007) were investigated. The prepared sequences underwent quantitative real-time PCR (qRT-PCR) analysis, utilizing a Bio-Rad® iQ5 fluorometric thermocycler. All primers (β-*actin, PPO1, Prx, p53, L5H*) were designed based on conserved sequences presented at GenBank, using Primer 5.0 software. Relative gene expression levels were quantified using the $2-\Delta\Delta^{Ct}$ method (Livak and Schmittgen, 2001). All qPCR reactions, inclusive of cDNA synthesis, were conducted in triplicate. To minimize data dispersion and promote a normal distribution, the qPCR data were transformed using a $\log^2$ function. Furthermore, the PCR amplification efficiency for each sample was performed using LinRegPCR software (Ramaker et al., 2003).

2.5 Statistical analysis

Earlier, before starting the statistical evaluations, the test of Levene's was applied to verify the data of normality as well as the assumption of homogeneity. Percentage values were transformed using an arc-sine function (Zar, 1984). The data are expressed as the mean followed by its respective standard deviation (SD). Statistical evaluations were made using SPSS Statistics Software (16.00), using one-way ANOVA, and then followed by Duncan's (Duncan, 1955) multiple range test under a $p < 0.05$. All figures were generated using GraphPad Prism 8 software (Swift, 1997). Correlation heatmap with cluster analysis was performed using Julius software.

3 Results

3.1 Water Quality, Growth and Feed Utilization Efficiency

There were no significant differences in water quality parameters between negative control group ($C_{0\%}$), positive control group ($SP_{2\%}$), and seaweed nanoparticle groups ($SN_{0.5\%}$, $SN_{1\%}$, and $SN_{2\%}$). Table 2 presents the survival, nutrient utilization, and growth indicators of shrimp reared in the experimental groups. No significant ($p < 0.05$) differences were observed in SR among all groups. The data indicated that shrimp reared on diets containing seaweed, whether in powder form (SP) or nanoparticle form (SN), exhibited significantly ($p < 0.05$) greater growth indices (SGR, WG, and FW) and nutrient efficiency (FI, PI, FER, FCR, and PER) comparing to the control group ($C_{0\%}$). Additionally, increasing the level of nanoparticles tended to enhance the improvement in the measured values (Table 2).

Figure 1 shows the polynomial regression chart between WG and FCR. The data presented in Figure 1 concludes that with the increase of SN dietary supplementation level, the polynomial regression of FCR was decreased ($r^2 = 0.9725$) and the polynomial regression of WG was increased ($r^2 = 0.8974$). From the findings presented in Figure 1, as determined by the polynomial regression model as a machine learning mode, it could be concluded

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TABLE 2 Growth and nutrient efficiency indicators of shrimp reared on the experimental dietary supplementation.

Indices	Groups				
	$C_{0\%}$	$SP_{2\%}$	$SN_{0.5\%}$	$SN_{1\%}$	$SN_{2\%}$
IW (g)	0.053 ± 0.002^{a}	0.054 ± 0.002^{a}	0.054 ± 0.001^{a}	0.054 ± 0.001^{a}	0.054 ± 0.001^{a}
FW (g)	3.950 ± 0.105^{d}	4.987 ± 0.116^{c}	4.950 ± 0.085^{c}	5.187 ± 0.045^{b}	5.400 ± 0.161^{a}
WG (g)	3.900 ± 0.105^{d}	4.933 ± 0.118^{bc}	4.900 ± 0.085^{c}	5.133 ± 0.050^{b}	5.347 ± 0.166^{a}
SGR (%/day)	3.337 ± 0.050^{c}	3.517 ± 0.015^{b}	3.510 ± 0.010^{b}	3.540 ± 0.017^{ab}	3.573 ± 0.032^{a}
FI (g)	7.807 ± 0.138^{c}	8.777 ± 0.010^{a}	8.370 ± 0.170^{a}	8.590 ± 0.050^{a}	8.727 ± 0.153^{a}
PI (g)	2.810 ± 0.053^{c}	3.157 ± 0.035^{a}	3.013 ± 0.123^{a}	3.090 ± 0.026^{a}	3.140 ± 0.056^{a}
FCR	2.007 ± 0.064^{a}	1.783 ± 0.045^{b}	1.707 ± 0.032^{bc}	1.673 ± 0.021^{c}	1.633 ± 0.067^{c}
FER	0.500 ± 0.016^{c}	0.562 ± 0.014^{b}	0.580 ± 0.011^{ab}	0.597 ± 0.008^{a}	0.613 ± 0.026^{a}
PER	1.387 ± 0.042^{c}	1.560 ± 0.040^{b}	1.623 ± 0.032^{ab}	1.660 ± 0.017^{a}	1.700 ± 0.072^{a}
SR (%)	85.30 ± 4.01^{a}	85.62 ± 3.05^{a}	87.50 ± 3.10^{a}	88.28 ± 3.02^{a}	86.50 ± 3.10^{a}

$C_{0\%}$, Control Basel diet; $SP_{2\%}$, Diet contains 2% (20 g/kg diet) powder of seaweed, as a positive control; $SN_{0.5\%}$, $SN_{1\%}$, and $SN_{2\%}$, Diets contain 0.5%, 1%, and 2% of seaweed nanoparticles (5, 10, and 20 g/kg diet, respectively). The data ($n = 5$) are the means ± SDs. Letters in the same column are significantly different ($p < 0.05$). IW, initial weight (g/shrimp); FW, Final weight (g/shrimp); WG, Weight gain (g/shrimp); SGR, Specific growth rate (%/day); PER, Protein efficiency ratio; PI, Protein intake; FER, Feed efficiency ratio; FCR, Feed conversion ratio; SR, Survival rate (%).

that the optimal range for SN supplementation is found to be between 1.2% and 1.6% (12–16 g/kg diet). This range corresponds to the $SN_{2\%}$ treatment group, where WG reached its highest peak and FCR achieved its lowest value.

3.2 Body carcass compositions

Table 3 reports the body carcass of shrimp reared on experimental diets. Table 3 indicated that shrimp reared on diets

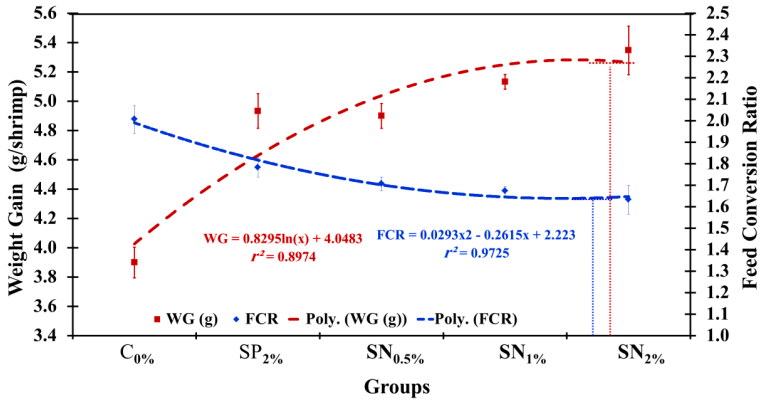

FIGURE 1
Polynomial regression chart between feed conversion rate and weight gain of shrimp reared on the experimental dietary supplementations. $C_{0\%}$, Control Basel diet; $SP_{2\%}$, Diet contains 2% (20 g/kg diet) powder of seaweed, as a positive control. $SN_{0.5\%}$, $SN_{1\%}$, and $SN_{2\%}$, Diets contain 0.5%, 1%, and 2% of seaweed nanoparticles (5, 10, and 20 g/kg diet, respectively). The data (n=5) were presented as means (± SD).

TABLE 3 Body carcass composition (%) of shrimp reared on the experimental dietary supplementation .

Composition (g/kg of dry matter basis)	Groups				
	$C_{0\%}$	$SP_{2\%}$	$SN_{0.5\%}$	$SN_{1\%}$	$SN_{2\%}$
Dry Matter	29.73 ± 0.62^{a}	26.17 ± 0.43b^{c}	26.81 ± 0.79^{b}	25.80 ± 0.40^{c}	24.80 ± 0.19^{d}
Lipid	4.37 ± 0.25^{c}	4.68 ± 0.09^{b}	4.88 ± 0.03^{b}	5.14 ± 0.06^{a}	4.87 ± 0.06^{b}
Protein	48.95 ± 0.83^{d}	51.03 ± 0.36^{c}	52.81 ± 0.12^{b}	53.07 ± 0.59^{b}	56.35 ± 1.40^{a}
Ash	12.67 ± 0.31^{a}	12.12 ± 0.25^{b}	12.35 ± 0.01^{ab}	12.20 ± 0.25^{b}	12.17 ± 0.12^{b}

$C_{0\%}$, Control Basel diet; $SP_{2\%}$, Diet contains 2% (20 g/kg diet) powder of seaweed, as a positive control; $SN_{0.5\%}$, $SN_{1\%}$, and $SN_{2\%}$, Diets contain 0.5%, 1%, and 2% of seaweed nanoparticles (5, 10, and 20 g/kg diet, respectively). The data (n=5) were presented as means (± SD), and the capital letters (a > b > c > d) mean significance values at $p < 0.05$.

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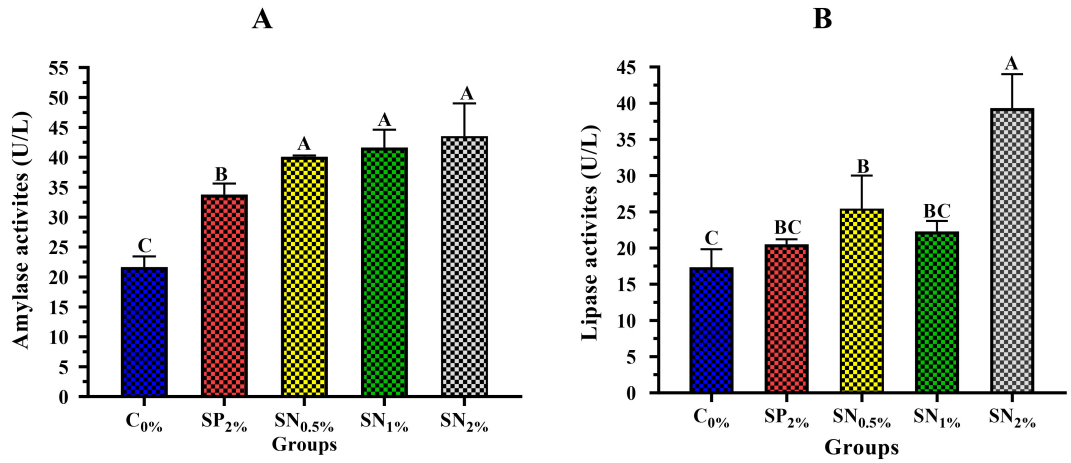

FIGURE 2
The activities of digestive enzyme levels of amylase **(A)** and lipase **(B)** of shrimp reared on the experimental dietary supplementation. $C_{0\%}$, Control Basel diet; $SP_{2\%}$, Diet contains 2% (20 g/kg diet) powder of seaweed, as a positive control; $SN_{0.5\%}$, $SN_{1\%}$, and $SN_{2\%}$, Diets contain 0.5%, 1%, and 2% of seaweed nanoparticles (5, 10, and 20 g/kg diet, respectively). The data (n=5) were presented as means (± SD), and the capital letters (A > B > C > D) mean significance values at $p < 0.05$.

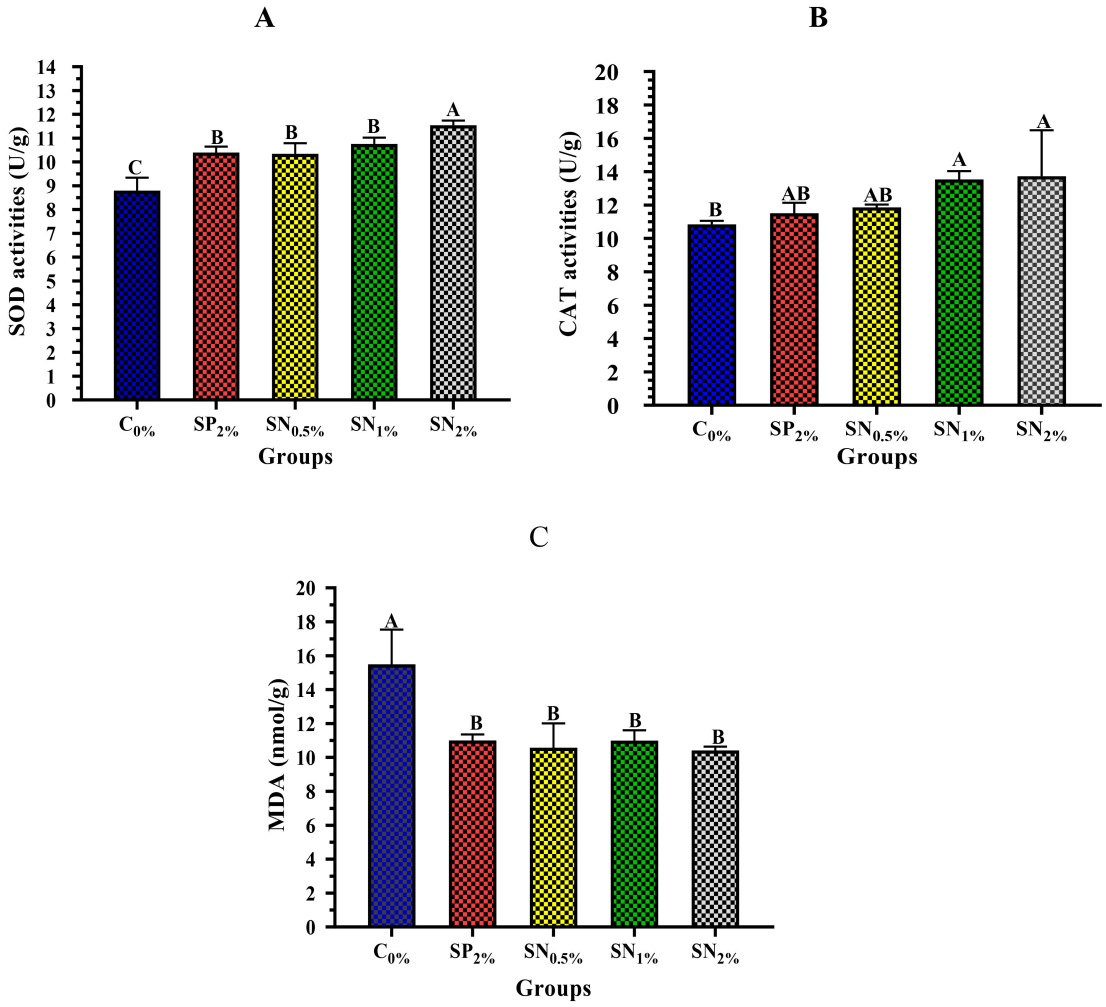

FIGURE 3
Antioxidant enzyme activities of SOD **(A)**, CAT **(B)**, and MDA **(C)** of shrimp reared on the experimental dietary supplementations. SOD, Serum superoxide dismutase (IU/g); CAT, Catalase (IU/g); MDA, Malondialdehyde (nmol/g); $C_{0\%}$, Control Basel diet; $SP_{2\%}$, Diet contains 2% (20 g/kg diet) powder of seaweed, as a positive control; $SN_{0.5\%}$, $SN_{1\%}$, and $SN_{2\%}$, Diets contain 0.5%, 1%, and 2% of seaweed nanoparticles (5, 10, and 20 g/kg diet, respectively). The data (n=5) were presented as means (± SD), and the capital letters (A > B > C > D) mean significance values at p <0.05.

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containing SN form ($SN_{0.5\%}$, $SN_{1\%}$, and $SN_{2\%}$) exhibited significant ($p < 0.05$) enhancement in lipid and protein percentages, followed by diets containing $SP_{2\%}$, compared to the control diet ($C_{0\%}$). Shrimp reared in group $SN_{2\%}$ showed the greatest significance protein (56.35%), while shrimp reared in group $SN_{1\%}$ showed the greatest significance lipid (5.14%). The highest ash (12.67%) and dry matter (29.73%) content was found in the control group ($C_{0\%}$), compared to either the SP or SN groups, as presented in Table 3.

3.3 Digestive enzyme activities

Figure 2 shows the impact of experimental dietary supplementations on shrimp digestive enzyme activities of amylase (Figure 2A) and lipase (Figure 2B).

From the presented data it could be concluded that the addition of SN to the shrimp diet significantly ($p < 0.05$) improved the shrimp's amylase and lipase activities higher than SP and the control diet. Compared to $C_{0\%}$ and $SP_{2\%}$, all SN groups ($SN_{0.5\%}$, $SN_{1\%}$, and $SN_{2\%}$) exhibited a higher significant value of amylase (Figure 2A), while $SN_{2\%}$ showed a higher significant value of lipase (Figure 2B).

3.4 Antioxidant enzymes activities

Figure 3 exhibits the effect of experimental dietary supplementations on shrimp's SOD level (Figure 3A), CAT level (Figure 3B), and MDA level (Figure 3C). The addition of SN to shrimp diets significantly ($p < 0.05$) enhanced the shrimp's SOD and CAT activities higher than SP and $C_{0\%}$. Shrimp reared in the SN2% group exhibited a higher significant ($p < 0.05$) value of SOD activity, followed by $SN_{1\%}$, $SN_{0.5\%}$, and $SP_{2\%}$, while the least significant of SOD value was reported in $C_{0\%}$ group (Figure 3A). On the other hand, shrimp reared in $SN_{2\%}$ and $SN_{1\%}$, groups showed highest values ($p < 0.05$) of CAT, followed by $SN_{0.5\%}$ and $SP_{2\%}$, while the least significant ($p < 0.05$) CAT value has reported in $C_{0\%}$ group (Figure 3B). On the other hand, shrimp reared in either SP or SN groups exhibit significantly ($p < 0.05$) lower values of MDA, noting that the lowest value was recorded in shrimp treated with the $SN_{2\%}$ (Figure 3C).

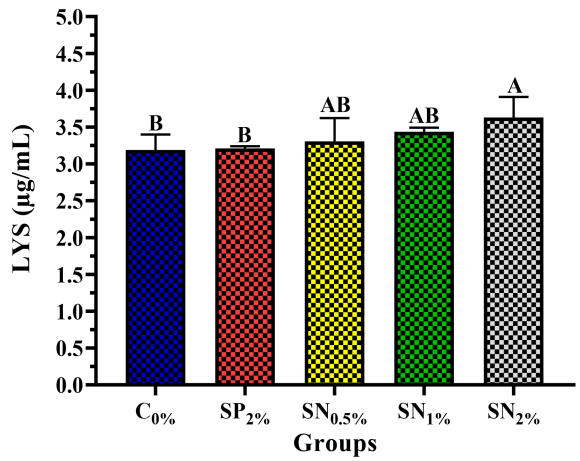

FIGURE 4
Innate immunity activities of LYS (A) of shrimp reared on the experimental dietary supplementations. LYS, Lysozyme (µg/mL); $C_{0\%}$, Control Basel diet; $SP_{2\%}$, Diet contains 2% (20 g/kg diet) powder of seaweed, as a positive control; $SN_{0.5\%}$, $SN_{1\%}$, and $SN_{2\%}$, Diets contain 0.5%, 1%, and 2% of seaweed nanoparticles (5, 10, and 20 g/kg diet, respectively). The data (n=5) were presented as means (± SD), and the capital letters (A > B > C > D) mean significance values at p <0.05.

3.5 Innate immunity activities

Figure 4 reflects the impact of experimental dietary supplementations on shrimp innate immunity enzyme activities of LYS (Figure 4). The groups containing SN ($SN_{0.5\%}$, $SN_{1\%}$, $SN_{2\%}$) significantly enhanced the shrimp's LYS activity higher than the SP and $C_{0\%}$ groups, noting that the highest value was reported in shrimp reared in the $SN_{2\%}$ group (Figure 4).

3.6 Immunity-related gene expressions

Figure 5 highlights the impact of experimental diets on shrimp's immunity-related gene expressions of *PRX* (Figure 5A), *PPO1* (Figure 4B), *p53* (Figure 4C), and *L5H* (Figure 4D). Figures 5A-D indicates that there is a significant difference ($p < 0.05$) in immunity-related gene expressions of shrimp reared in groups containing SP or SN, compared to $C_{0\%}$. Compared to the $C_{0\%}$ group, the *PRX* gene expression was significantly ($p < 0.05$) upregulated in shrimp reared in group $SN_{1\%}$ followed by $SN_{2\%}$, whereas it was not upregulated in other groups (Figure 5A). Figure 5B shows that compared to the $C_{0\%}$ group, the gene expression of *PPO1* was significantly ($p < 0.05$) upregulated in shrimp reared in group $SN_{0.5\%}$, followed by $SN_{1\%}$, $SN_{2\%}$, and $SP_{2\%}$.

Figure 5C shows that the *p53* gene expression was significantly ($p < 0.05$) increased in shrimp reared in group $SN_{2\%}$, followed by $SN_{1\%}$, $SN_{0.5\%}$, and $C_{0\%}$, while the lowest significant ($p < 0.05$) decrease was achieved by $SP_{2\%}$ group. The highest significant ($p < 0.05$) increase in *L5H* gene expression was obtained by shrimp reared in the control group $C_{0\%}$, followed by shrimp reared in $SP_{2\%}$, $SN_{0.5\%}$, $SN_{2\%}$, while the lowest significant decrease was achieved by $SN_{1\%}$ group (Figure 5D).

3.7 Correlation heatmaps

Figure 6 shows the correlation heatmap analysis with cluster heat map (Figure 6A) and circular heatmap (Figure 6B). Furthermore, association analysis of treatments and estimated variables showed that18 variables out of 23 falls into cluster 1 with a mean association value of 4.96. Cluster 2 contains just 1 variable, but with a much higher means association (86.64). Cluster 3 has 4 variables, with a mean association of 35.33. This also revealed a dose dependent manner of increasing seaweed *P. capillacea* nanoparticles supplementation.

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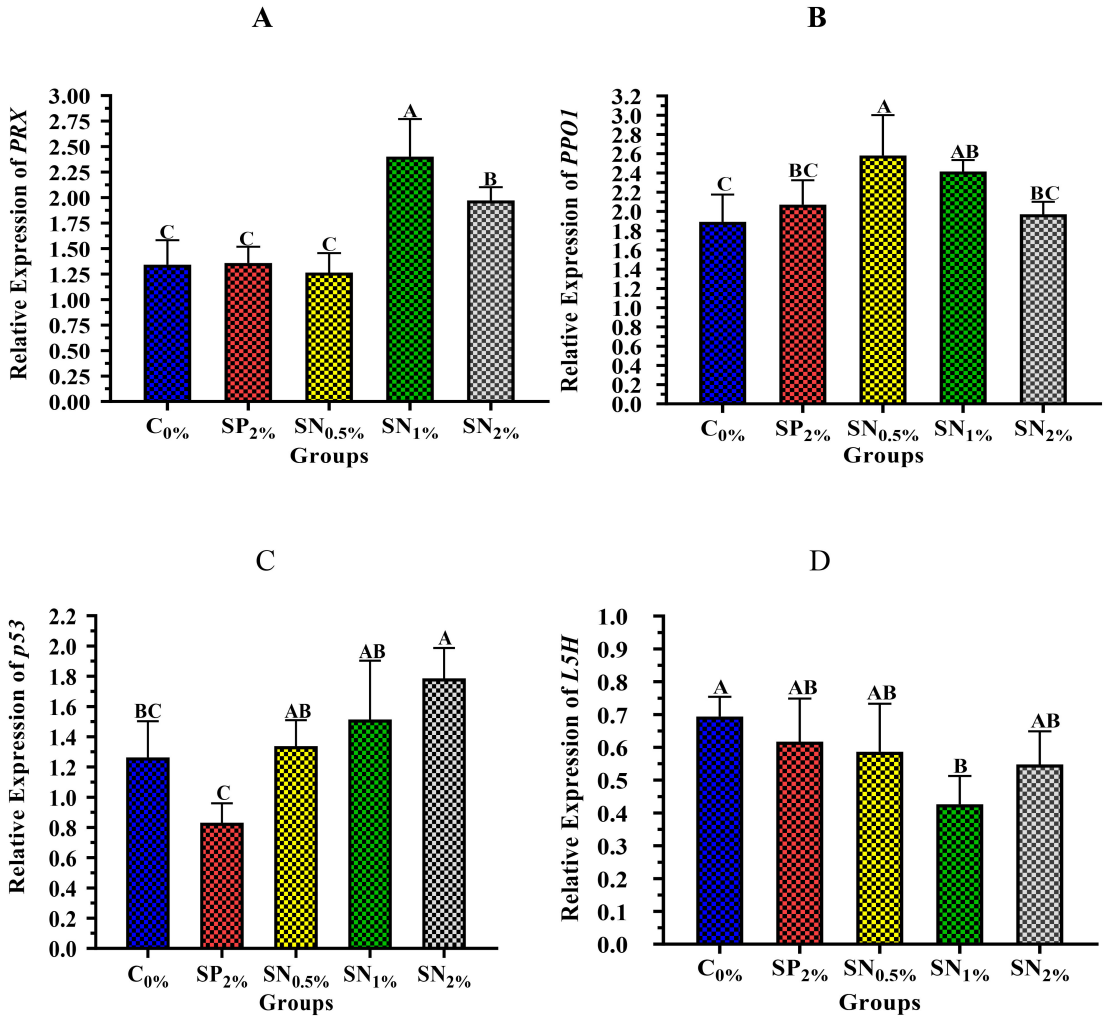

FIGURE 5
Immunity-related gene expressions of shrimp reared on the experimental dietary supplementations: **(A)** peroxiredoxin gene, *Prx*; **(B)** prophenoloxidase gene, *PPO1*; **(C)** p53-like protein isoform delta gene, *p53*; and **(D)** hemocyanin subunit L5 gene, *L5H*. $C_{0\%}$, Control Basel diet; $SP_{2\%}$, Diet contains 2% (20 g/kg diet) powder of seaweed, as a positive control, $SN_{0.5\%}$, $SN_{1\%}$, and $SN_{2\%}$, Diets contain 0.5%, 1%, and 2% of seaweed nanoparticles (5, 10, and 20 g/kg diet, respectively). The data (n =5) were presented as means (± SD), and the capital letters (A > B > C > D) mean significance values at $p < 0.05$.

4 Discussion

Applying nanotechnology tools to aquafeed diets-based algal cells (microalgae and seaweed) significantly maximizes the benefit of their impact on aquatic animals (Mabrouk et al., 2022). However, according to the best of our knowledge, this is the first study in the literature conducted to determine the role of red seaweed *P. capillacea* nanoparticles on overall improvement in the shrimp *L. vannamei* postlarvae production. The current investigation revealed that, compared with those of the positive or negative controls, with the increasing dietary supplementation levels of SN, the growth (FW, WG, and SGR), digestive enzymes (amylase and lipase activities), carcass composition (protein and lipid contents), nutrient efficiency (FI, PI, FER, FCR, and PER), antioxidant activities (SOD and CAT), innate immunity activities (LYS and MDA), and related-immunity gene expressions (*p53*) of *L. vannamei* were significantly improved. The findings of the current work were confirmed by the findings recorded by Sharawy et al. (2022), who concluded that the implementation of *A. platensis* nanoparticles as a feed additive in the *L. vannamei* postlarvae regime significantly led to improvements in growth achievements, enhanced biochemical composition, and promoted growth-related gene (*IGF-I* and *IGF-II*) expressions.

In the same line, Mabrouk et al. (2022) highlighted that supplementing Nile tilapia diets, exposed to *Aeromonas hydrophila*, with the nanoparticles of *A. platensis* resulted in notable improvements in blood chemistry of total protein, albumin, and globulin levels. In addition, the antioxidant markers (SOD, CAT, GPx, lysozyme activity, and respiratory burst) were substantially elevated in fish fed diets containing these doses compared to both negative and positive controls. Additionally, it significantly decreased serum glucose levels and the enzymatic activities of ALT and AST. However, dietary supplementation with *A. platensis* nanoparticles

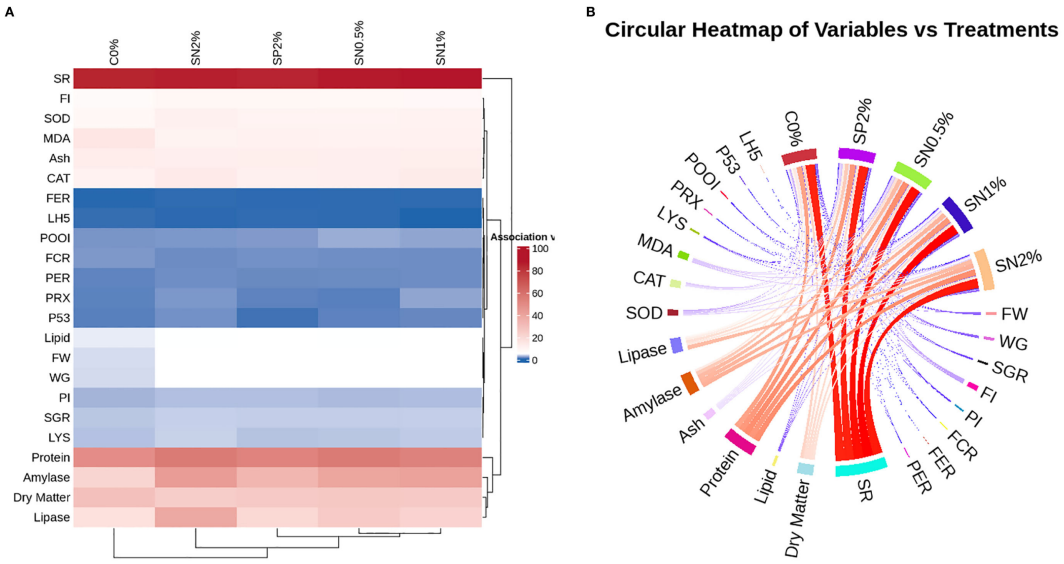

FIGURE 6
The association of treatments and estimated variables in a clusted heat map **(A)** or circular heatmap **(B)**. The color scale ranges from blue (low association) through white (medium) to red (high association).

effectively mitigated the inflammation in kidney, liver, and spleen tissues that was caused by *A. hydrophila* (Mabrouk et al., 2022).

In aquaculture, FCR and WG are the most common indicators that can judge the quality of diets. FCR is a ratio of feed input to WG (Wan et al., 2019). It explains how efficiently shrimp can convert feed ingredients into body mass gain. A lower FCR is desirable (Weber et al., 2025). Shrimp aquafeed additives play a vital role in improving both FCR and WG in shrimp diets.

In the current work, the polynomial regression chart between FCR ($r^2 = 0.9725$) and WG ($r^2 = 0.8974$) demonstrates that when SN dietary supplementation levels rise, the polynomial regression of FCR was decreased while WG was increased. As reported previously by Khanjani et al. (2024), these findings reflect that, by enhancing nutrient absorption and growth promotion, these additives contribute to more efficient and sustainable shrimp farming practices. Our finding are consistent with those published by Ashour et al. (2025), who noted that polynomial regression analysis of FCR was significantly reduced and WG was significantly increased when the inclusion supplementation levels of cyanobacteria *D. tharense* NIOF17/006 in the diet of shrimp *L. vannamei* postlarvae were increased.

Regarding the shrimp's body carcass compositions, the current findings are consistent with those conducted by Abbas et al. (2023), who recorded significant variations in the overall body composition of postlarvae of shrimp *L. vannamei* when they were fed diets containing varying amounts of polysaccharide derived from *S. dentifolium*. Furthermore, our results aligned with earlier research by Sharawy et al. (2022) and Mabrouk et al. (2022) that employed *A. platensis* nanoparticles as feed additives for *L. vannamei* and *O. niloticus* diets, respectively.

The activity of digestive and antioxidant enzymes serves as valuable indicators for evaluating feed additive quality (Ashraf et al., 2024). These correlate to enhanced nutrient uptake, efficient digestion, and thus superior health in shrimp (Liu et al., 2024). Our study revealed that with the increasing dietary SN supplementation levels, the digestive enzymes and antioxidant activities significantly improved, resulting in improvement in nutrient absorption, digestion, and overall shrimp health quality. As reported by our previous work conducted by Ashour et al. (2020), *P. capillacea* has a respectable amount of bioactive compounds (polysaccharide, phenolic, flavonoid, carotene, PUFA, MOFA, EAA, *etc.*, Supplementary Table S1), which showed several biological activities, including antioxidant activities. Our findings were highlighted by Hu et al. (2025), who reported that the utilization of natural antioxidant substrates as feed additives in shrimp aquadiets promotes overall shrimp health quality. The current findings may be due to the content of bioactive compounds and other functional groups present in the utilized red seaweed species. The same findings were supported by several authors when using red seaweed species, in all their forms, as feed additives for shrimp, *L. vannamei* (Abbas et al., 2023); Oscar, *Astronotus ocellatus* (Khanzadeh et al., 2024); *O. niloticus* (Shahabuddin et al., 2024), Northern snakehead, *Channa argus* (Wang et al., 2025); rainbow trout, *Oncorhynchus mykiss* (Sotoudeh and Mardani, 2018); rabbit fish, *Siganus canaliculatus* (Bakky et al., 2023); and hybrid red tilapia (Abdelrhman et al., 2022).

Innate immunity is a valuable key for assessing shrimp's aquafeed additive efficiency (Chen et al., 2024; Kim et al., 2024). Since shrimp don't have an adaptive immune system, their innate immunity acts as their first defense mechanism (Rathinam et al., 2024). In shrimp, LYS represent crucial indicators of innate immunity (Saha et al., 2024). MDA, a lipid peroxidation outcome, is an indicator of oxidative stress and the effectiveness of shrimp's antimicrobial defense system. Lower levels of MDA are generally encouraged (Yan et al., 2025). The ability of the innate immune system to fight off infections is reflected by LYS.

Higher lysozyme activity is often encouraged (Chen et al., 2025). To evaluate how aquatic feed additives promote disease resistance and overall shrimp wellness, MDA and LYS work together by providing essential data on the immune system and overall wellness of shrimp (Abdollahzadeh et al., 2025). Our findings showed that with the increasing of SN dietary supplementation, the LYS activities were significantly increased while MDA decreased. These findings reveal that the addition of SN in shrimp diet (as a natural antioxidant substrate) positively improves the innate immunity and, consequently, improves the defense mechanism in shrimp (Hu et al., 2025). These results may be attributed to the detected amount of bioactive compounds in red seaweed *P. capillacea* (Ashour et al., 2020), in addition to the properties of nanoparticles that are characterized in *P. capillacea* (Mansour et al., 2022b).

Our investigation showed that incorporating SN into shrimp diets enhanced the expression of immune-related genes, in particular *Prx*, *PPO1*, and *p53*, while no improvement was obtained in *L5H*. These observations align with the previous study by Zhu et al. (2011), who demonstrated that supplementing *L. vannamei* diets with *P. capillacea* led to reduced inflammation, improved immune enzyme activity, and increased expression of immune-related genes. These findings can be the consequence of the bioactive substances (Ashour et al., 2020), as well as the characterization of *P. capillacea* nanoparticles (Mansour et al., 2022b). Immune-related gene expression in *L. vannamei* was significantly enhanced by supplementing their diets with *P. capillacea* polysaccharide (Abbas et al., 2023), a combined seaweed extract (Ashour et al., 2024a), and *A. platensis* free-lipid (Ashour et al., 2024b).

5 Conclusion

Utilizing nanotechnology tools to enhance shrimp feed additives can significantly maximize the positive effects of these additives, leading to improvements in *L. vannamei* growth, health, and overall production. Our findings indicate that incorporating nanoparticles of red seaweed *P. capillacea* at levels up to 2% (20 g/kg of feed) markedly boosts growth, immune function, antioxidant capacity, nutrient efficiency, digestive enzyme activity, and the expression of genes associated with immunity in *L. vannamei*. Moreover, the polynomial regression model concluded that the optimal range for SN supplementation is found to be between 1.2% and 1.6% (12–16 g/kg diet). This range corresponds to the $SN_{2\%}$ treatment group, where WG reached its highest peak and FCR achieved its lowest value. Further research is required to understand and explain how seaweed nanoparticles affect these shrimp's physiological state and upgrade some immune-related gene expressions.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

MA: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. MM: Conceptualization, Data curation, Investigation, Methodology, Supervision, Writing – original draft. AIM: Conceptualization, Investigation, Methodology, Validation, Writing – original draft. MN: Software, Writing – original draft. ATM: Data curation, Formal analysis, Funding acquisition, Investigation, Software, Supervision, Validation, Writing – review & editing. EM: Resources, Software, Supervision, Writing – review & editing. AA: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2025.1600260/full#supplementary-material

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