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Evaluation of caper (*Capparis spinosa*) extract supplementation on growth performance, antioxidant activity, innate immunity, immune-related gene expression, and resistance to *Vibrio parahaemolyticus* in *Litopenaeus vannamei*

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Capparis spinosa L. (caper) is a drought-resistant shrub valued for its therapeutic, pharmaceutical, and culinary properties. This study evaluated the biological activities of its aqueous (AQE) and methanolic (MEE) extracts on growth performance, innate immunity, antioxidant activity, immune-related gene expression, and resistance to *Vibrio parahaemolyticus* in *Litopenaeus vannamei*. Aqueous and methanolic extracts were prepared and analyzed, demonstrating that the methanolic extract of *C. spinosa* (MEE) exhibited significantly stronger antioxidant activity ($P < 0.05$) in terms of total phenolic content (TPC), ferric reducing antioxidant power (FRAP), and DPPH scavenging activity than the aqueous extract (AQE). According to HPLC analysis, RP-HPLC for phenolic component characterization in *C. spinosa* L. extractions identified 16 phenolic compounds; sinapic acid predominated in MEE, while only trace gentisic and benzoic acids were noticed in AQE, with all the other phenolics being absent from AQE. Overall, MEE exhibited substantially higher phenolic content than AQE. For feeding/challenge test: A three-month nourishment experiment was performed to investigate the impact of supplementing the diet with AQE and MEE of *C. spinosa* at concentrations of 1.0, 2.0, and 3.0% kg^{-1} feed. Juvenile shrimp 1.66 ± 0.55 g (N = 2100) were randomly distributed into 21 fiberglass tanks (250 L), with three replicates per treatment, and fed the experimental diets. Diets containing higher MEE levels (3.0% kg^{-1}) significantly ($P<0.05$) improved growth performance, feed utilization, immune parameters, antioxidant activities, and expression of immune-related genes, particularly hemocyanin (Hc) and penaeidin-3a (Pen-3a) genes compared with other groups. Following the *V. parahaemolyticus* challenge, shrimp fed the

3.0% MEE diet showed the highest survival (75%; P< 0.05). These results indicate that adding 3.0% methanolic extract of *C. spinosa* to the diet enhances growth, immunological status, antioxidant response, and resistance to infection in juvenile *L. vannamei*.

KEYWORDS

methanolic extracts, feed efficiency, hepatopancreas morphometric, body composition, *L. vannamei*

1 Introduction

The global shrimp aquaculture industry has expanded dramatically, now accounting for approximately 7–8% a dominant share of world aquaculture production (Dongyu, 2024; Hassan et al., 2023). The Pacific whiteleg shrimp (*Litopenaeus vannamei*) dominates aquaculture, representing 83.1% of worldwide shrimp output due to its elevated survival rates, fast development, and resilience to diseases (Kumar et al., 2025). This species has advantageous characteristics for breeding selectively, which include a low dietary protein requirement, resilience to high stocking densities, extensive environmental adaptability, and simplicity of adaptation (Emerenciano et al., 2022). Despite these advantages, *L. vannamei* farming faces persistent challenges, including low disease survival (e.g., vibriosis), substandard water condition, and ecological stressors (El-Sayed, 2021; Emerenciano et al., 2022). Mounting antibiotic resistance from overuse of synthetic chemicals underscores the urgent need for eco-friendly alternatives that bolster shrimp immunity (Locatelli et al., 2017). Dietary immunostimulants enhance growth performance, immune responses, and pathogen resistance while mitigating environmental stressors (Kord et al., 2021). Shrimp immunity relies exclusively on innate defenses, encompassing cellular (Roy et al., 2025) and humoral mechanisms that detect and eliminate pathogens (Sharawy et al., 2022). Effective feed additives can be evaluated via antioxidant activities, immunological indices, immune-related gene expression, and reduced oxidative stress (Hu, 2022; Zhang et al., 2022). Plant-derived compounds, rich in secondary metabolites like flavonoids, offer potent antimicrobial, antioxidant, and immunomodulatory properties (Ashour et al., 2024). Caper (*Capparis spinosa* L.), traditionally used in ancient Egyptian and Arab medicine, exemplifies such a resource (Tlili et al., 2011). Its flower buds contain bioactive phenolics (Zagórska-Dziok et al., 2025), that confer strong DPPH radical-scavenging and antioxidant effects via enzymatic and non-enzymatic systems neutralizing reactive oxygen species (Seferli et al., 2024). Supplementing 2.0 g/kg with caper ethanolic extracts shows significant antibacterial activity against *Streptococcus agalactiae*, improves growth performance, immunological and antioxidant responses, and disease resistance in Nile tilapia, and improves survival after bacterial challenge (Abdel-Razek et al., 2023). In rainbow trout (*Oncorhynchus mykiss*), dietary supplementation with *C. spinosa* methanolic extract (0.1–0.5 g kg^{-1} feed) was linked to a moderated enhancement of innate immune responses and cytokine gene expression, which was linked to improved growth performance and increased trout survival after *Aeromonas hydrophila* challenge (Bilen et al., 2016). No research has clearly distinguished or systematically examined its impact and functional role in shrimp nutrition and health. Therefore, the current investigation assessed the impact of supplementing the diet with aqueous and methanolic *C. spinosa* extracts at inclusion levels of 1.0, 2.0, and 3.0% on *L. vannamei*, focusing on growth efficiency, nutrient utilization, immunity, antioxidant activity, immune-gene expression, and resistance to *Vibrio parahaemolyticus*.

2 Materials and methods

2.1 Ethical statement

The experiment was done following the ethical considerations, adhering to the guidelines established by the institution and the relevant ethics committee for animal care at the King Salman International University, El-Tor, Egypt. Approval number: KSIU/2026/DA-3.

2.2 Reagents, chemicals, and instrumentation

Tokyo Chemical Industry (TCI) (Tokyo, Japan) and Thermo Fisher Scientific (Waltham, MA, USA) were the sources of phenolic benchmarks with a purity of over 99%. Fluka Chemie GmbH of Buchs, Switzerland, supplied the trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), TPTZ, and Folin-Ciocalteu phenol reagent, while Sartorius of Göttingen, Germany, supplied the polytetrafluoroethylene (PTFE) membranes. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Tokyo Chemical Industry (TCI) (Tokyo, Japan) with 95% purity. Commercial assay kits for phenoloxidase, superoxide dismutase, catalase, and malondialdehyde were acquired from Biodiagnostic and/or Biotechnology Co. (Egypt). All remaining reagents were of analytical quality and obtained from local scientific suppliers in Egypt.

2.3 Bacterial and yeast strains

Conventional and molecular methods were employed to verify the isolation of *V. parahaemolyticus* from diseased shrimp supplied by the Faculty of Veterinary Medicine, Alexandria University, Egypt according to (Nishio et al., 2015) methodology. For challenge tests post-feeding trial, the isolate was cultured in tryptic soy broth (TSB) supplemented with 1.5% NaCl (pH 7.5, 30°C, 48 h) (Joshi et al., 2014). *Micrococcus lysodeikticus* (lyophilized, 0.2 mg mL^{-1}) served

as the substrate for lysozyme activity assays by being cultured in nutrient broth and incubated at 30°C for 24 h under aerobic conditions. The yeast *Candida albicans* strain was employed for phagocytic activity determination by culturing on Sabouraud dextrose broth (SDB) and incubating at 28–30°C for 24–48 h.

2.4 Plant material

Collecting aerial pieces of *C. spinosa* L. took place in April 2025 at Ras El Hekma, Egypt. Taxonomic identification was confirmed by experts in the Botanic Garden & Herbarium, Alexandria University. The freshly collected material was shade-dried in a dark, well-ventilated area, then stored in clean, light- and moisture-proof bags before extraction.

2.5 Preparation of *C. spinosa* extract

Aqueous extracts of *C. spinosa* leaves were prepared from material washed with tap and distilled water, shade-dried for 5-10 days, and kept in a sealed polyethylene case at 5°C pending processing. Leaves were ground to powder, and 20 g was infused in 200 mL boiling distilled water for 15 min, filtered, concentrated to dehydration, and preserved at 4°C (Algandaby et al., 2014). Extraction yield was calculated as follows:

$$\% \ Y = (Me)/(SM) \times 100$$

where Me is the mass of the extracted material, and SM is the starting mass of the leaf powder.

Methanolic extract was prepared by soaking the ground dried *C. spinosa L.* leaves in 400 ml of methanol for 48 h. Following the soaking process, the alcohol was eliminated using a rotary evaporator, and the extracted concentrate was utilized to provide the needed concentrations for further studies (El-Darier and Abdelhady, 2017).

2.6 Determination of antioxidant capacity

According to Akyuz et al. (2014), the Folin-Ciocalteu technique was used to estimate the total phenolic content (TPC) using gallic acid as a standard. TPC was computed using a standard curve (0.015 – 0.5 mg mL^{-1}; $r^2 = 0.99$).

The ferric reducing antioxidant power (FRAP) assay was performed to measure the sample's antioxidant capacity via ferric (Fe^{3+}) to ferrous (Fe^{2+}) reduction, following Benzie and Strain (1996) with minor modifications.

The radical scavenging activity of *C. spinosa* L. extracts against the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical was evaluated spectrophotometrically at 517 nm (Molyneux, 2004). Butylated hydroxytoluene (BHT) was used as a benchmark to quantify radical scavenging activity.

According to Kao et al. (2011), the flavonoid concentration was measured using a modified aluminum chloride colorimetric test. The findings were presented in mg, with a mean ± SD and a sample size of 3.

2.7 The chemical composition of liquid extracts derived from *C. spinosa* L. leaves

Phenolic substances were examined via high-performance liquid chromatography (HPLC) utilizing an Agilent 1100 series system. Eluates were analyzed at 270-280 nm, with peaks recognized based on comparable retention durations and regions in comparison to benchmarks.

2.8 Feeding trial

Seven iso-nitrogenous (40.70% crude protein), iso-lipidic (14.20%), and iso-caloric (17.43 MJ kg^{-1} gross energy) trial feeds were formulated. A basal control diet (CONT 0%) received no *C. spinosa* supplementation, while diets 2-4 contained aqueous extract (AQE) at 1.0, 2.0, and 3.0%, and diets 5–7 contained methanolic extract (MEE) at equivalent levels (1.0, 2.0, and 3.0%). Dry ingredients were blended homogeneously, pelleted (2mm diameter) using the Amandus Kahl Germany Laboratory Pellet Mill at <40°C, and the extract levels 1.0, 2.0, and 3.0% Kg^{-1} feed were dissolved with 100 mL distilled water and then sprayed onto the pellets; the control received water alone. Feeds were dried at 45°C for 48 h (moisture< 10%), coated with 5 mL kg^{-1} sunflower oil, air-dried for 4 h, sealed in plastic containers, and preserved at 4°C pending use. The chemical composition of the ingredients and the control feed done according to (AOAC, 2000) and is displayed in Table 1; gross energy was calculated per Jobling (1983).

2.9 Culture technique and water quality

The experiment was conducted at a commercial marine shrimp farm in Borg El Arab, Alexandria Province, Egypt. *Litopenaeus vannamei* postlarvae (PL_{23}; initial weight 1.75 ± 0.25 g) sourced from the Ghalioun Project hatchery (Egypt) were first acclimated for 21 days in two 2000-L fiberglass tanks, fed the control diet (40% crude protein) until reaching uniform size. Subsequently, 2100 juveniles were graded and stocked into 21 × 250-L fiberglass tanks (100 shrimp $tank^{-1}$; density 400 shrimp m^{-3}; 3 replicates $treatment^{-1}$; 7 treatments) for a 12-week trial under a 12:12 h light: dark photoperiod. Water quality variables were: salinity 26 ± 1 ppt, temperature 28 ± 2°C, dissolved oxygen (DO) ≥ 5 mg L^{-1} (continuous aeration), pH 8.25 ± 0.35, NH_3 0.04 ± 0.01 mg L^{-1}, NO_2^- 0.08 ± 0.01 mg L^{-1}, NO_3^- 0.12 ± 0.02 mg L^{-1} (Boyd and Tucker, 2012). Tanks received ~10% daily water exchange via pond inflow/outflow, with regular siphoning. Shrimp were given diets to satiation three times daily. For challenge tests, treatments were transferred to 75-L glass aquaria (3 tanks per $treatment^{-1}$; acclimation 2 weeks beforehand) under matching conditions (temperature 28 ± 2°C, DO ≥ 5 mg L^{-1}, salinity adjusted from 40 ppt groundwater to 26 ± 1 ppt).

2.10 Growth response and feed efficiency

At the beginning and end of the feeding trial, postlarval body weight was measured using a digital analytical balance. The mean

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TABLE 1 Approximately the chemical structure and composition of the basic meal (based on dry matter).

Formulation & proximate composition	Experimental diets formulation (g/kg)
Fish meal (72.0% CP)	175.00
Fish processing waste	175.00
Soybean meal (45.0% CP)	250.00
Wheat gluten (76% CP)	110.00
Rice bran (defat) (21.7% CP)	95.00
Corn meal (8.3% CP)	65.00
Licithin	10.00
Corn oil	30.00
Fish oil	30.00
Vitamins Premix[1]	10.00
Minerals Premix[2]	10.00
Vitamin C	1.00
Starch	9.00
Dicalcium phosphate	30.00
Total	**1000.00**
Proximate composition (%)	
Dry matter	85.94
Crude Protein	45.94
Total lipids	14.20
Fiber	2.67
Ash	10.53
NFE	26.63
Gross Energy (MJ/kg)	17.43
Gross Energy (kcal/100 gm)	511.04

[1]Vitamin Premix (kg^{-1} of diet): riboflavin, 450 mg; cyanocobalamin, 1.00 mg; menadione, 39 mg; biotin, 8 mg; myo-inositol, 7500 mg; nicotinic acid 190 mg; vitamin A, 230 000 IU; pyridoxine HCL, 390 mg; thiamin, 19 mg; folic acid, 120 mg; vitamin D, 35 000 IU; a-tocopherol, 2500 IU; calcium pantothenate, 690 mg; choline chloride 7500 mg.
[2]Minerals Premix (kg^{-1} of diet): $CaCO_3$, 190 g; KI, 0.03 g; $CuSO_4$ $5H_2O$, 2.9 g; $FeSO_4$-$7H_2O$, 19 g; $MgSO_4$ $7H_2O$, 120 g; $ZnSO_4$ $7H_2O$, 3.5 g; KCL, 85 g; NaCl, 35 g; $CoSO_4$ $7H_2O$, 0.01 g; $MnSO_4$ H_2O, 2.5 g; Ca $(HPO_4)2$ $2H_2O$, 450 g.

initial body weight was calculated from five postlarvae per replicate, with three replicates per treatment. At the end of the experimental period, the final body weight was recorded. Based on these measurements, several growth performance indices were calculated using the subsequent formulas:

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

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

$$\text{Feed Conversion Ratio (FCR)} = (\text{Total consumed feed})/(\text{WG});$$

$$\text{Feed Efficiency Ratio (FER)} = (\text{WG(g)})/(\text{Feed intake (g)});$$

$$\text{Protein Efficiency Ratio (PER)} = (\text{WG(g)})/(\text{Protein intake (g)}).$$

2.11 Samples of hemolymph and tissues

At the end of feeding trials, shrimp were fasted for 24 h. Five shrimp were randomly collected from each tank (15 per treatment), and hemolymph was withdrawn from the ventral sinus and divided into two sets. The first one was allowed to coagulate at 4 °C for 24 h, then centrifuged (2000 × g, repeated) to obtain aliquoted serum, which was stored at -20°C. The second aliquot (750 µL) was transferred into a precooled (4°C) anticoagulant solution composed of 0.114 M trisodium citrate, 450 mM NaCl, 10 mM KCl, and 10 mM HEPES (pH 7.4) (Nonwachai et al., 2010). Hepatopancreas tissues were excised, rapidly frozen in liquid nitrogen, and preserved at -80°C for gene expression and biochemical examinations.

2.11.1 Immune response parameters

To quantify total hemocyte counts (THCs), hemolymph was added to anticoagulant solution within a Neubauer chamber (Smith and Soderhall, 1983). Hemocyte count was estimated utilizing a hemocytometer following gathering hemolymph.

THC (cells/mL)= count $\times 10^4$ ×dilution factor. Phagocytic activity was measured using the Selvin et al. (2004) methods. Hemocytes have been identified at 200 per sample. To measure phagocytic activity, use a formula as follows: Phagocytosis (%) = (Number of phagocytic hemocytes)/(Total number of hemocytes) ×100. According to the turbidimetric approach outlined by Engstad et al. (1992), the activity of lysozyme (LYZ) was assessed by following the equation: Lysozyme activity=(A0-A)/A.

After making some adjustments to an existing technique, the spectrophotometric measurement of phenoloxidase activity was taken by keeping track of the amount of dopachrome that was formed from l-dihydroxyphenylalanine (Supamattaya and Pongmaneerat, 2000).

2.11.2 Antioxidant enzyme activity and lipid peroxidation

According to McCord and Fridovich (1969), superoxide dismutase (SOD) activity was measured. By adding 10 µL of serum to the kit reagent and monitoring the breakdown of H_2O_2, the activity of catalase (CAT) was determined spectrophotometrically. Malondialdehyde (MDA), a sign of lipid peroxidation, was measured using the thiobarbituric acid-reactive substances (TBARS) method (Ohkawa et al., 1979).

2.11.3 Immune-related genes expression

The procedures used for these studies were conducted in accordance with the methodologies delineated by Aguilera-Rivera et al. (2019). The reaction was performed at 37°C for 80 minutes. Afterwards, qPCR with specific primers for the hemocyanin (Hc) and penaeidin-3a (Pen-3a) genes was conducted according to the procedure outlined in an earlier work, with the gene β-actin serving

TABLE 2 Primers for real-time quantitative RT-PCR.

Gene name	Primer sequence (5′- 3′)	Annealing temperature	Product (bp)	Accession no or references
β-actin	Forward primer:5′- GTGCCCATCTACGAGGGATA-3′	56.5°C	233	AF300705
	Reverse primer:5′-TAGGACTTCTCCAGCGAGGA-3′			
Hc	Forward primer:5′-GTCTTAGTGGTTCTTGGGCTTGTC-3′	37°C	124	(Aguilera-Rivera et al., 2019)
	Reverse primer:5'-GGTCTCCGTCCTGAATGTCTCC-3'			
Pen-3a	Forward primer:5'-CTGGTCTTCTTGCCTCCTT-3'	37°C	121	AF390139
	Reverse primer:5′-ATATCCCTTTCCCACGTGAC-3′			

β-actin, beta actin (a housekeeping gene); Hc, hemocyanin and Pen-3a, penaeidin-3a genes.

as the internal control (Wang et al., 2007). All of this is detailed in Table 2. The relative expression of each gene was determined using the $2^{-\Delta\Delta Ct}$ equation (Schmittgen and Livak (2008), where Ct is the value that corresponds to the cycle number during which the fluorescence was produced.

2.11.4 Bacterial challenge with *V. parahaemolyticus*

After 12 weeks of experimental feeding, fifteen shrimp were chosen from each aquarium and placed in a brand-new 75-L water glass aquarium (three aquariums per group). A 100 µL volume of *V. parahaemolyticus* was prepared by culturing on Thiosulphate Citrate Bile Salt Sucrose agar (TCBSA) and incubated at 28°C for 48 h at a bacterial density of 10^8 cfu mL^{-1} and inoculated between the muscles halfway through the third abdominal section in all groups (Noble et al., 2017). Each treatment represented 45 of *L. vannamei* shrimp. The experimental design was summarized as follows:

T1: Shrimp were given a basal feed (control feed) that was non-enriched with both (AQE) and (MEE) and not challenged with *V. parahaemolyticus*;

T2: Basal feed (control feed) was non-enriched with both (AQE) and (MEE) and challenged with bacterial densities of 10^8 cfu mL^{-1} *V. parahaemolyticus*;

T3, T4, T5: Shrimp were given a basal feed enriched with 1.0, 2.0, and 3.0% of AQE of *C.spinosa*, respectively, and challenged with bacterial densities of 10^8 cfu mL^{-1} *V. parahaemolyticus*;

T6, T7, T8: Shrimp were given a basal feed enriched with 1.0, 2.0, and 3.0% of MEE of *C. spinosa*, respectively, and challenged with *V. parahaemolyticus* densities of 10^8 cfu mL^{-1}.

Shrimps were monitored daily for clinical symptoms and death. Following bacterial infection, shrimp were computed after 7 days from challenges as detailed below:

$$\text{Total Cumulative Mortality (TCM) in shrimp} = (\text{Total deaths} \div \text{Initial stocked shrimp}).$$

$$\text{Mortality}(\%) = (\text{TCM} \div \text{Initial number of stocked shrimp}) \times 100.$$

$$\text{Relative percent survival RPS}\% = \left(1 - \frac{\%\,\text{MTest}}{\%\,\text{MControl}}\right) \times 100.$$

Where M is a mortality in the test group and control.

2.12 Hepatopancreas morphometric analysis

Histopathological lesions were evaluated using a semi–quantitative scoring approach to reduce subjectivity and enhance reproducibility (Table 3). For each shrimp, tissue sections were prepared following standard protocols and examined under light microscopy. Assessment procedure: Five shrimp per treatment, three non-overlapping visual fields per shrimp (cephalothorax, abdomen, and appendages) and scoring performed independently by two blinded observers.

Hepatopancreas histopathology analyses from 5 shrimp per tank (across *C. spinosa* aqueous and methanolic extract treatments) were collected post-*V. parahaemolyticus* challenge at week 12. After 48 h of fixation in Davidson's fluid, the tissues were transferred to 70% ethanol and then dehydrated using xylene and graded ethanol (70%, 80%, 95%, 100%). To distinguish between healthy and infected tissues, the samples were fixed in paraffin, cut into 5 µm sections, stained with hematoxylin and eosin (H&E), and then studied under a microscope at 10× and 40× magnification.

2.13 Statistical analysis

Statistics are expressed as means ± standard error (n = 5). The data were arcsine-transformed before analysis, and Levene's test was utilized to verify normality and homogeneity of variance. A

TABLE 3 Gross lesion severity score (GLS).

Score	Criteria (external appearance)
0	Normal morphology, no visible lesions
1	Very mild discoloration or localized redness
2	Mild–moderate redness, slight melanization, limited appendage involvement
3	Moderate lesions affecting multiple regions (muscle opacity, focal necrosis)
4	Severe extensive lesions (widespread melanization, hemorrhage, deformity

one-way ANOVA and Duncan's multiple-range test (P< 0.05) were performed using SPSS 23.00.

3 Results

3.1 *In vitro* total antioxidant capacity of extract of *Capparis spinosa L.*

Total phenolic compounds (TPC) were quantified relative to standard gallic acid Figure 1.

shows that the methanolic extract of *C. spinosa* L. has a stronger total phenolic compound (TPC) and ferric reducing antioxidant power (FRAP) activity than the aqueous extract (P< 0.05). The total phenolic content (TPC) of the aqueous extract was 27.52 ± 0.13, while that of the methanolic extract was 38.38 ± 0.09 mg GAE 100 g^{-1} DW. Higher antioxidant activity was seen in the methanolic extract, as shown by a FRAP of 142.07 ± 0.14, compared to 119.07 ± 0.19 in the aqueous extract. The DPPH radical scavenging activity was 0.17 ± 0.02 and 0.29 ± 0.03 SC_{50}: mg. mL^{-1} in the aqueous extract and methanolic extract of *C. spinosa L.*, respectively. As shown in Figure 1, the sample extract can scavenge hydroxyl radicals by donating hydrogen atoms or electrons in the DPPH test.

3.2 RP-HPLC for phenolic component characterization in *C. spinosa* L. extractions

RP-HPLC examination identified 16 phenolic compounds in *C. spinosa* L. extracts (Supplementary Figure 1 in Supplementary Materials): protocatechuic acid, gallic acid, chlorogenic acid, gentisic acid, protocatechuic aldehyde, p-hydroxybenzoic acid, vanillin, syringic acid, caffeic acid, rosmarinic acid, vanillic acid, syringaldehyde, p-coumaric acid, benzoic acid, sinapic acid, and ferulic acid. Sinapic acid predominated in the MEE, while only trace gentisic and benzoic acids were noticed in the AQE; all other phenolics were absent from AQE. Overall, MEE exhibited substantially higher phenolic content than AQE.

3.2.1 Growth performance

Dietary supplementation with 3.0% MEE *C. spinosa* L. extracts significantly enhanced (P< 0.05) growth rate and survival in *L. vannamei* in comparison with the control (Table 4). Optimal responses occurred at higher doses, particularly MEE, with no differences (P > 0.05) among AQE 1.0, 2.0%, and MEE 1.0%. Treatment with MEE 3.0% yielded the highest specific growth rate (SGR) and survival rates, exceeding AQE 2.0% (78.1%), AQE 1.0% (77.7%), and control (68.2%).

3.2.2 Feed efficiency and productivity metrics

L. vannamei intake *C. spinosa* L. extract-supplemented diets displayed substantially (P< 0.05) improved feed utilization and productivity versus the unsupplemented control (Table 5). Extract treatments reduced feed intake, enhanced feed conversion ratio (FCR), and increased protein efficiency ratio (PER). Peak productivity occurred in MEE 3.0%.

Results are presented using average ± SE. The averages in the columns marked by various superscripts within a parameter are significantly (P< 0.05) different from each other, n = 5. Treatments: A basal control diet (CONT 0%) received no *C. spinosa* supplementation, while diets contained aqueous extract at (AQE 1.0, 2.0, and 3.0%) and diets contained methanolic extract (MEE) at equivalent levels (MEE 1.0, 2.0, and 3.0%).

3.2.3 Immune response parameters

Non-specific immune parameters-total hemocyte count (THC), phagocytic activity, lysozyme (LYZ), and phenoloxidase (PO)

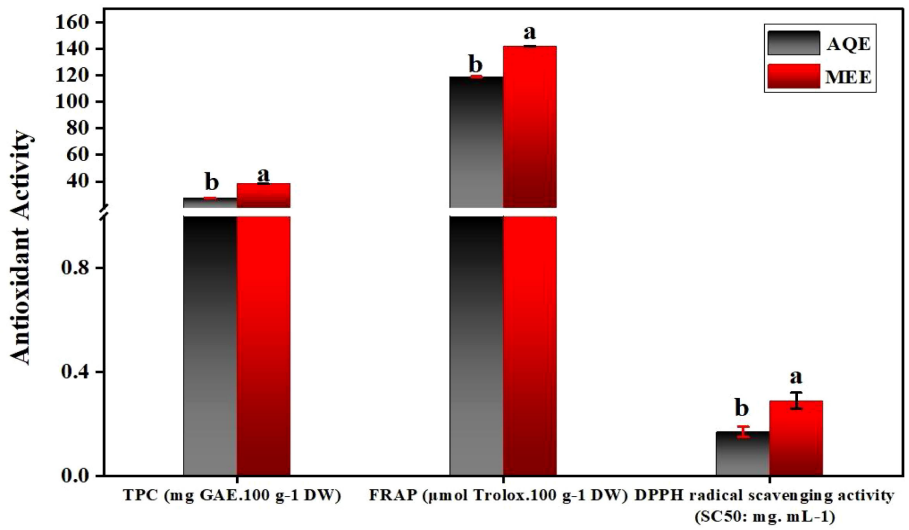

FIGURE 1
Antioxidant activities of aqueous extract (AQE) and methanolic extract (MEE) of *Capparis spinosa L.* Values are expressed as the mean ± standard error of triplicate groups. Values with various superscript letters in one column are substantially dissimilar (P< 0.05). TPC, Total phenolic compounds; FRAP, ferric reducing antioxidant power; DPPH, 2,2-diphenyl-1-Picrylhydrazyl.

TABLE 4 Growth and survival rate of *L. vannamei* intake *Capparis spinosa* supplementation under an intensive culture system.

Treatments	Growth and survival parameters							
	IW	FW	WG	ADG	AWG	SGR	RGR	Survival (%)
$CONT_{0\%}$	1.73 ± 0.03	12.80 ± 0.03 ^{e}	11.07 ± 0.50 ^{e}	0.131 ± 0.01 ^{e}	0.92 ± 0.02 ^{f}	2.38 ± 0.06 ^{e}	738.8 ± 6.18 ^{e}	80.33 ± 0.71 ^{g}
$AQE_{1.0\%}$	1.66 ± 0.05	13.72 ± 0.02 ^{d}	12.05 ± 0.02 ^{d}	0.14 ± 0.02 d	1.00 ± 0.01 ^{e}	2.51 ± 0.07 ^{d}	824.8 ± 17.58 ^{d}	83.67± 1.17 ^{f}
$AQE_{2.0\%}$	1.70 ± 0.06	14.62 ± 0.02 ^{bc}	12.92 ± 0.03 ^{d}	0.15 ± 0.02 ^{bc}	1.07± 0.01 ^{d}	2.56 ± 0.08 ^{bc}	860.8 ± 12.77 ^{bc}	87.00 ± 0.60 ^{e}
$AQE_{3.0\%}$	1.66 ± 0.07	15.33 ± 0.58 ^{bc}	13.58 ± 0.47 ^{bc}	0.16 ± 0.03 ^{bc}	1.13± 0.05 ^{b}	2.64 ± 0.06 ^{bc}	921.9 ± 9.56 ^{bc}	91.67 ± 0.60 ^{c}
$MEE_{1.0\%}$	1.70 ± 0.07	14.57 ± 0.32 ^{bc}	12.87 ± 0.32 ^{bc}	0.15 ± 0.04 ^{bc}	1.07 ± 0.03 ^{c}	2.55± 0.03 ^{bc}	858.2 ± 14.68 ^{bc}	88.00 ± 0.60 ^{d}
$MEE_{2.0\%}$	1.70 ± 0.07	15.92 ± 0.17 ^{b}	14.22 ± 0.02 ^{b}	0.16 ± 0.05 ^{b}	1.18± 0.04 ^{ab}	2.66 ± 0.08 ^{b}	936.7 ± 17.87 ^{b}	93.00 ± 0.751 ^{b}
$MEE_{3.0\%}$	1.68 ± 0.06	17.82 ± 0.23 ^{a}	16.13 ± 0.02 ^{a}	0.19 ± 0.073 ^{a}	1.25± 0.02 ^{a}	2.80± 0.05 ^{a}	1060.9 ± 18.68 ^{a}	97.00 ± 1.335 ^{a}

IW, Initial weight, g/shrimp^{3}, FW, Final weight, g/shrimp, WG, Weight gain, g/shrimp, ADG, Average daily gain g / shrimp/day, AWG, Average weekly gain g / shrimp/week, SGR, Specific growth rate % / shrimp /day, RGR, Relative growth rate% / shrimp. Treatments: A basal control diet (CONT 0%) received no *C. spinosa* supplementation, while diets contained aqueous extract at (AQE 1.0, 2.0, and 3.0%) and diets contained methanolic extract (MEE) at equivalent levels (MEE 1.0, 2.0, and 3.0%). Results are presented using average ± SE. The averages in the columns marked by various superscripts within a parameter are significantly (P< 0.05) different from each other, n = 5.

activity are concise in Figure 2. Shrimp intake methanolic *C. spinosa* L. extract (MEE) diets displayed substantially improved (P< 0.05) THC, LYZ, PO, and phagocytosis versus controls. Overall, both AQE and MEE treatments enhanced immunity (P< 0.05) relative to unsupplemented shrimp, with peak THC and phagocytosis in AQE 3.0%, MEE 2.0, and 3.0%. Maximum LYZ and PO occurred in MEE 2.0, and 3.0%, confirming *C. spinosa* extracts' efficacy in boosting *L. vannamei* immunity.

3.2.4 Antioxidant activity and lipid peroxidation

Shrimp fed MEE diets exhibited significantly higher (P< 0.05) in hemolymph catalase and superoxide dismutase activities than those fed AQE or control diets (Figure 3). Conversely, MDA concentration, an indicator of lipid peroxidation, was dramatically decreased (P< 0.05) in all extract treatments compared with controls. Peak antioxidant enzyme elevation and MDA reduction occurred in MEE 3.0%, with methanolic supplementation showing highly significant effects (P< 0.05).

3.2.5 Immune-related gene expressions

Figure 4 exhibited the mRNA transcription of the Hc and Pen-3a genes in shrimp intake feed enriched with *C. spinosa* L. extracts, and increased methanolic extract levels significantly increased Hc and Pen-3a gene transcription with MEE 3.0% treatment. Generally, gene expression of Hc and Pen-3a was substantially upregulated (P< 0.05) as the level of methanolic *C. spinosa* L. extracts in the feed increased in comparison to the control.

TABLE 5 Feed efficiency and productivity metrics of *L. vannamei* fed of *Capparis spinosa* supplemented diet under a system of an intensive culture.

Treatments	Feed efficiency and productivity metrics		
	Feed Intake, g/ shrimp	FCR	PER, g/g
$CONT_{0\%}$	23.72 ± 0.22 ^{f}	2.14 ± 0.02 ^{e}	1.16 ± 0.01 ^{e}
$AQE_{1.0\%}$	23.48 ± 0.11 ^{f}	1.94 ± 0.01 ^{d}	1.28 ± 0.01 ^{de}
$AQE_{2.0\%}$	21.33 ± 0.14 ^{e}	1.66 ± 0.01 ^{c}	1.51 ± 0.01 ^{d}
$AQE_{3.0\%}$	19.43 ± 0.01 ^{d}	1.38 ± 0.02 ^{c}	1.80 ± 0.01 ^{cd}
$MEE_{1.0\%}$	20.77 ± 0.04 ^{cd}	1.60 ± 0.01 ^{b}	1.55 ± 0.01 ^{bc}
$MEE_{2.0\%}$	18.90 ± 0.03 ^{b}	1.36 ± 0.01 ^{b}	1.83 ± 0.01 ^{b}
$MEE_{3.0\%}$	15.67 ± 0.01 ^{a}	0.97 ± 0.02 ^{a}	2.59 ± 0.01 ^{a}

3.2.6 Bacterial challenge

3.2.6.1 Rates of death and survival

Results of the challenge with *V. parahaemolyticus* on *L. vannamei*, including the relative survival rate and cumulative death rate (Figure 5). Following seven days the negative control group (T1) showed negligible mortality, whereas the infected control (T2) exhibited the highest TCM and mortality and the lowest RPS, confirming the severe pathogenic effect of *V. parahaemolyticus* (Figure 5). *L. vannamei* fed diets containing MEE displayed more pronounced protective effects than AQE, with lower TCM and mortality and higher RPS values across all corresponding inclusion levels.

Overall, both extracts enhanced shrimp resistance to *V. parahaemolyticus* infection, with the highest survival and lowest mortality observed in shrimp receiving the 3.0% MEE level.

3.2.6.2 Clinical signs

Following *V. parahaemolyticus* challenge, clinical signs differed markedly between the control group and shrimp fed diets supplemented with AQE or MEE extracts of *C. spinosa* L. (Supplementary Figure 2, Supplementary Materials). Positive control shrimp exhibited severe clinical signs and post–mortem lesions, including lethargy, anorexia, reduced movement, and pronounced black discoloration of the hepatopancreas by the second day post–infection. These symptoms progressively worsened throughout the trial, with affected shrimp becoming weak, settling at the tank bottom, showing enlarged chromatophores, and ultimately exhibiting pale, atrophied hepatopancreas and empty digestive tracts. In contrast, shrimp fed AQE (2.0 and 3.0%) exhibited moderate pathological changes at 7 days post–challenge, including whitish abdominal muscle discoloration, softened muscle texture, faded shells, and

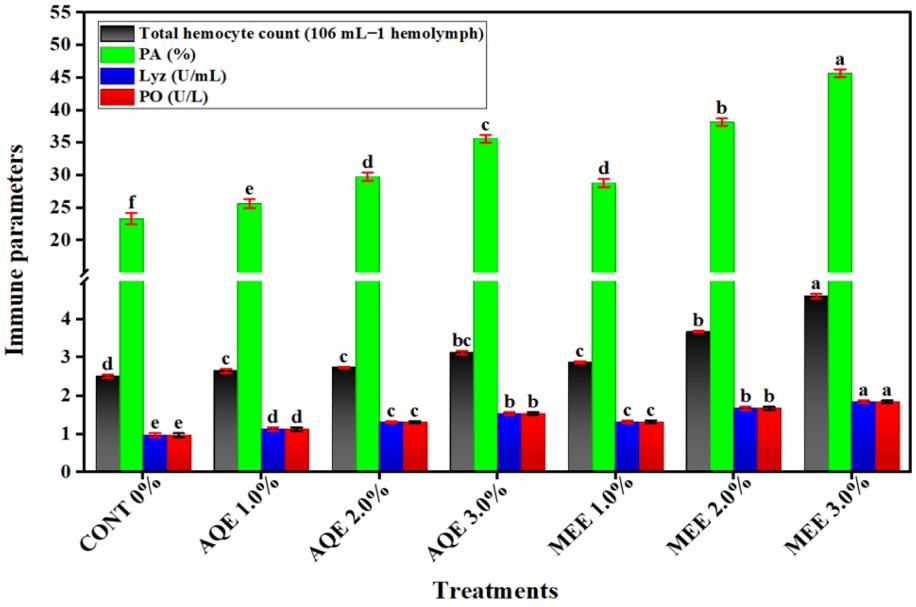

FIGURE 2
Immune parameters of *L. vannamei* fed a *Capparis spinosa* supplemented diet under an intensive culture system. PA, Phagocytosis activity; Lyz, Lyzozyme activity; PO, Phenoloxidase activity. Results are presented using average ± SE. The averages in the columns marked by various superscripts within a parameter are significantly (P< 0.05) different from each other, n = 5. Treatments: A basal control diet (CONT 0%) received no *C. spinosa* supplementation, while diets contained aqueous extract at (AQE 1.0, 2.0, and 3.0%) and diets contained methanolic extract (MEE) at equivalent levels (MEE 1.0, 2.0, and 3.0%).

mild tail fan necrosis. Shrimp fed higher levels of MEE (2.0 and 3.0%) showed only slight clinical abnormalities, such as reduced activity, dullness, and occasional impaired swimming.

Gross lesion severity (GLS) scores differed markedly among treatments following *V. parahaemolyticus* infection (Table 6). Uninfected shrimp in the negative control group showed no visible lesions (GLS = 0.0), whereas the infected control group exhibited severe pathological signs, including pronounced redness, melanization, and multiple lesions, with the highest GLS score (4.0). Dietary supplementation with *C. spinosa* L. extracts reduced lesion severity in a dose–dependent manner. Shrimp fed AQE diets showed progressive reductions in GLS scores from 3.0 - 2.0 at 1.0 -3.0% AQE treatments. However, MEE supplementation resulted in stronger protective effects, with GLS scores decreasing

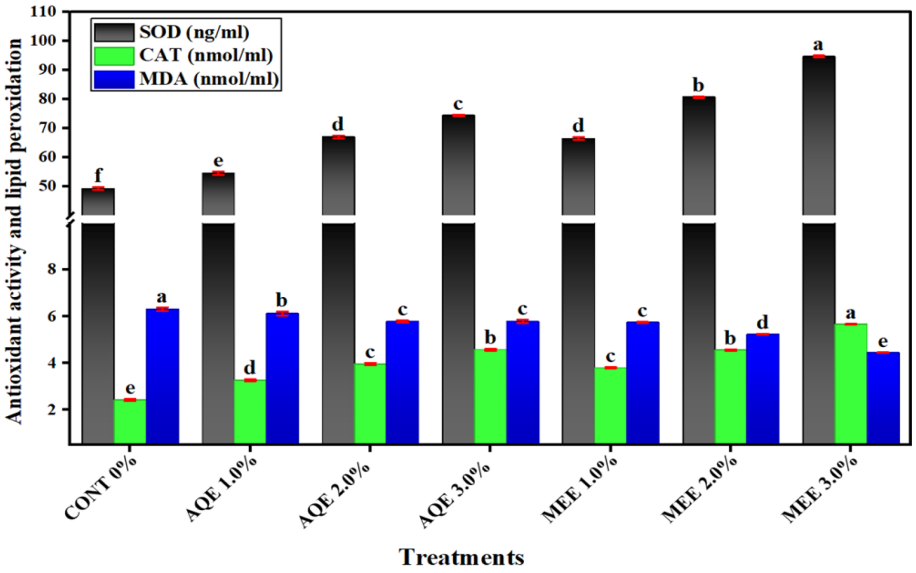

FIGURE 3
Antioxidant activity and lipid peroxidation parameters of *L. vannamei* fed a *C. spinosa* supplemented diet under an intensive culture system. SOD, superoxide dismutase activity; CAT, Catalase activity; MDA, malondialdehyde levels. Results are presented using average ± SE. The averages in the columns marked by various superscripts within a parameter are significantly (P< 0.05) different from each other, n = 5. Treatments: A basal control diet (CONT 0%) received no *C. spinosa* supplementation, while diets contained aqueous extract at (AQE 1.0, 2.0, and 3.0%) and diets contained methanolic extract (MEE) at equivalent levels (MEE 1.0, 2.0, and 3.0%).

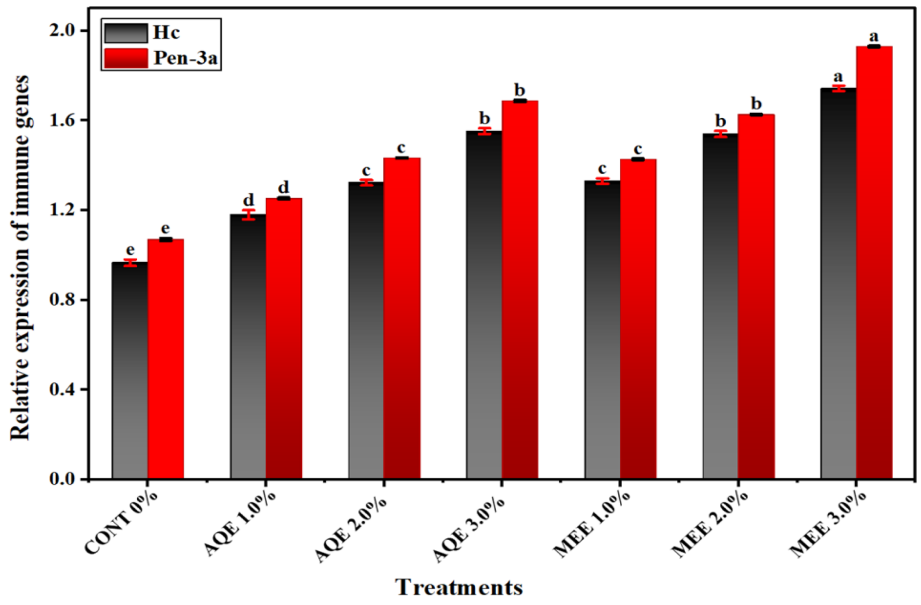

FIGURE 4
Relative expression of immune genes of Pacific whiteleg shrimp, *Litopenaeus vannamei*, fed a *C. spinosa* L. enriched diet under a system of intensive culture for 12 weeks.Hc, hemocyanin gene; Pen-3a, penaeidin-3a gene. Results are presented using average ± SE. The averages in the columns marked by various superscripts within a parameter are significantly (P< 0.05) different from each other, n = 5. Treatments: A basal control diet (CONT 0%) received no *C. spinosa* supplementation, while diets contained aqueous extract at (AQE 1.0, 2.0, and 3.0%); and diets contained methanolic extract (MEE) at equivalent levels (MEE 1.0, 2.0, and 3.0%).

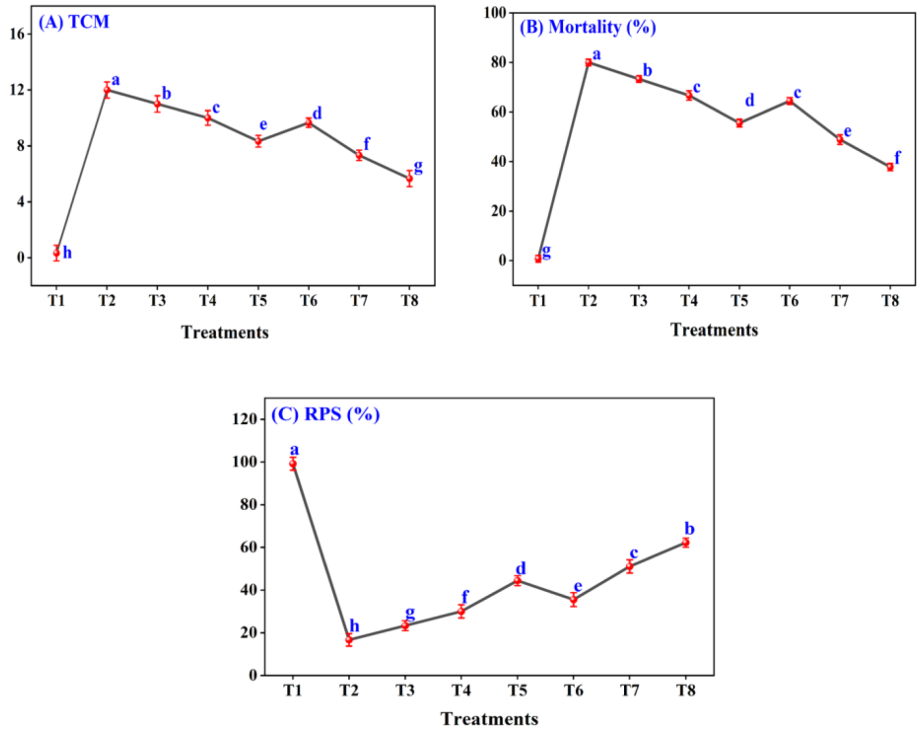

FIGURE 5
Rates of death and survival; TCM, total cumulative mortality, mortality and RPS, Relative percent survival of *L. vannamei* infected with *V. parahaemolyticus* after feeding a *C. spinosa L.* extracts with different levels. Results are presented using average ± SE. The averages in the lines marked by various superscripts within a parameter are significantly (P< 0.05) different from each other, n = 5. Treatments: T1: A basal control diet (- C. *spinosa* - V. *parahaemolyticus*), T2: A basal control diet (- C. *spinosa* +*V. parahaemolyticus*), T3, T4, T5: (+ 1.0, 2.0, and 3.0% of AQE *C. spinosa* +*V. parahaemolyticus*), T6, T7, T8: (+ 1.0, 2.0, and 3.0% of MEE *C. spinosa* +*V. parahaemolyticus*).

TABLE 6 Gross lesion severity scoring of *L. vannamei* infected with *V. parahaemolyticus* after feeding of *C. spinosa L.* extracts with different levels.

Treatment and description	Mean GLS score
Control (–*C. spinosa*–*V. parahaemolyticus*): normal shrimp	0.00 ± 0.00
Infected control: severe redness, melanization, multiple lesions	4.00 ± 0.00
1.0% AQE + infection; moderate-severe focal lesions	3.00 ± 0.20
2.0% AQE + infection; moderate localized lesions	2.50 ± 0.20
3.0% AQE + infection; mild lesions, reduced melanization	2.00 ± 0.10
1.0% MEE + infection; moderate lesions	2.50 ± 0.20
2.0% MEE + infection; mild-moderate lesions	1.50 ± 0.10
3.0% MEE + infection; minimal visible lesions	1.00 ± 0.10

from 2.5 at 1.0% MEE to 1.0 at 3.0% MEE, indicating minimal visible lesions. Overall, highest inclusion level 3.0% MEE, particularly, were associated with marked attenuation of gross lesion severity and clinical signs.

3.2.7 Hepatopancreas morphometric analysis after infected shrimp with *V. parahaemolyticus*

Histopathological examination revealed marked variations in hepatopancreatic lesions among shrimp groups (Table 7; Figure 6). Non-infected shrimp fed non-supplemented diets exhibited normal hepatopancreatic architecture with well-organized blind tubules, narrow lumens, and intact epithelial layers composed of F-, E-, B-, and R-cells (T1). In contrast, shrimp challenged with *V. parahaemolyticus* and fed non-supplemented diets showed severe pathological alterations, including tubular atrophy, epithelial sloughing, necrosis, epithelial degeneration, and hemocyte infiltration (T2).

Dietary supplementation with 1.0% kg^{-1} AQE caused noticeable deformation and atrophy of B- and R-cells with irregular tubule organization, whereas higher AQE levels (2.0 and 3.0% kg^{-1}) resulted in only mild structural alterations (T3–T4). Similarly, shrimp fed 1.0% kg^{-1} MEE displayed irregular hepatopancreatic structures with damaged epithelial membranes and distorted lumens (T5). However, higher MEE levels (2.0 and 3.0% kg^{-1}) improved tissue organization, showing intact epithelial membranes and regularly arranged tubules with well-developed cells (T6 - T7). Moreover, *V. parahaemolyticus* was detected at lower levels in shrimp receiving both extracts, particularly at higher supplementation levels, compared with the positive control. These findings indicate that dietary *C. spinosa* extracts may enhance hepatopancreatic recovery and *L. vannamei* resistance to *V. parahaemolyticus* infection. The dietary supplementation with 3.0% kg^{-1} MEE caused noticeable improvement of all histopathological changes induced by experimental infection of *V. parahaemolyticus* (T8). The research also included tubular atrophy, epithelial sloughing, necrosis, epithelial degeneration, and hemocyte infiltration tubules. Since the F-, E-, B-, and R-cells, which are considered the main hepatopancreatic architecture, and its tubule organization are important in the immunity of shrimp.

4 Discussion

C. spinosa L. (caper) compounds, rich in secondary metabolites like flavonoids, offer potent antimicrobial, antioxidant, and immunomodulatory properties. The total phenolic content (TPC) of aqueous extract was lower than methanolic extract, while higher antioxidant activity was seen in the methanolic extract compared to the aqueous extract. Several authors, including Tlili et al. (2011), have reported that *C. spinosa* L. is abundant in phenolic components.

Both extracts have been assessed for their capacity to neutralize radicals that are unstable by applying the DPPH test. The *C. spinosa* L. extracts were more effective than butylated hydroxytoluene (BHT), a common antioxidant, when tested against other conventional antioxidants. This enhanced activity may be attributed to the significant variation in polyphenol content and their corresponding radical scavenging capacities. The aqueous extract showed higher DPPH radical scavenging activity (SC_{50} = 0.17 ± 0.02 mg·mL^{-1}) compared with the methanolic extract (SC_{50} = 0.29 ± 0.03 mg·mL^{-1}). Both extracts demonstrated hydroxyl radical scavenging capacity, indicating their ability to donate hydrogen or electrons in the

TABLE 7 Hepatopancreatic morphometric analysis of white-leg shrimp feeding extracts of *C. spinosa L.* with different levels for 12 weeks and challenged shrimp with *V. parahaemolyticus*.

Hepatopancreas morphometric analysis	Treatments							
	T1	T2	T3	T4	T5	T6	T7	T8
HI	0.00 ± 0.0 ^{g}	100.0 ± 0.0 ^{a}	91.67 ± 1.37 ^{b}	75.00 ± 0.0 ^{c}	58.33 1.33 ^{d}	50.00 ± 0.0 ^{e}	50.00 ± 0.0 ^{e}	25.00 ± 0.0 ^{f}
Lack of B, R, and F cells	0.00 ± 0.0 ^{g}	100.0 ± 0.0 ^{a}	91.67 ± 1.37 ^{b}	75.00 ± 1.80 ^{c}	66.67 ± 1.33 ^{d}	50.00 ± 0.0 ^{e}	25.00 ± 0.0 ^{f}	25.00 ± 0.0 ^{f}
SHP	0.00 ± 0.0 ^{f}	100.0 ± 0.0 ^{a}	91.67 ± 1.29 ^{b}	75.00 ± 0.00 ^{c}	75.00 ± 1.13 ^{c}	50.00 ± 0.0 ^{d}	50.00 ± 0.0 ^{d}	25.00 ± 0.0 ^{e}
EN	0.00 ± 0.0 ^{g}	100.0 ± 0.0 ^{a}	91.67 ± 1.37 ^{b}	75.00 ± 1.80 ^{c}	66.67 ± 1.33 ^{d}	50.00 ± 0.0 ^{e}	25.00 ± 0.0 ^{f}	25.00 ± 0.0 ^{f}
B	0.00 ± 0.0 ^{f}	100.0 ± 0.0 ^{a}	75.00 ± 1.67 ^{b}	75.00 ± 1.80 ^{b}	66.67 ± 1.33 ^{c}	50.00 ± 0.0 ^{d}	25.00 ± 0.0 ^{d}	25.00 ± 0.0 ^{e}

HI, Hemacytes infiltration; SHP, Sloughing of hepatopancreatic tissues; EN, Enlargement of nucleus; B, presence of bacteria in hepatopancreatic tissues. Results are presented using average ± SE. The averages in the rows marked by various superscripts within a parameter are significantly (P< 0.05) different from each other, n = 5. Treatments: T1: A basal control diet (- C. *spinosa* - V. *parahaemolyticus*), T2: A basal control diet (- C. *spinosa* +*V. parahaemolyticus*), T3, T4, T5: (+ 1.0, 2.0, and 3.0% of AQE *C.spinosa* +*V. parahaemolyticus*), T6, T7, T8: (+ 1.0, 2.0, and 3.0% of MEE *C. spinosa* +*V. parahaemolyticus*).

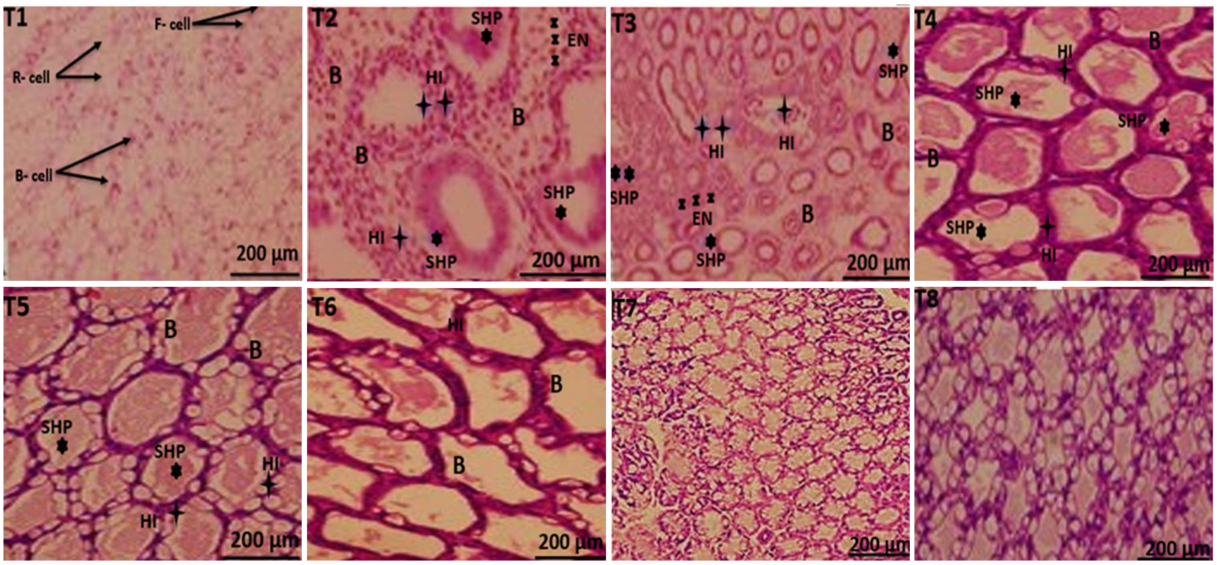

FIGURE 6
Hepatopancreas morphometrics of white-leg shrimp (*L. vannamei*) feeding on *C. spinosa L.* with different levels of supplemented diet for 12 weeks post-challenge intramuscularly with *V. parahaemolyticus*. B-cell, R-cell, F cell; HI, Hemacytes infiltration; SHP, Sloughing of hepatopancreatic tissues; EN, Enlargement of nucleus; B: presence of bacteria in hepatopancreatic tissues. Stain H and (E) Bar = 200 µm. All images were calibrated prior to scale bar insertion; scale bars represent identical absolute distances (µm), independent of microscope magnification, (n = 5). Treatments: T1, A basal control diet (-*C. spinosa* -*V. parahaemolyticus*); T2, A basal control diet (- C. *spinosa* +*V. parahaemolyticus*); T3, T4, T5, (+ 1.0, 2.0, and 3.0% AQE *C. spinosa* + *V. parahaemolyticus*); T6, T7, T8, (+1.0, 2.0, and 3.0% MEE *C. spinosa* + *V. parahaemolyticus*).

DPPH assay. The hydrogen-donating ability of phenolic compounds suggests their role as free radical scavengers (El Finou et al., 2024; Molyneux, 2004). ROS such as hydrogen peroxide and hydroxyl radical are potent chemical byproducts naturally generated during cellular energy production (Bakr and El Bishbishy, 2016; Mollica et al., 2019). In the DPPH assay, the extracts' capacity to scavenge hydroxyl radicals reflects their hydrogen- or electron-donating potential. Al-Asady et al. (2012) reported higher IC_{50} values, indicating lower antioxidant activity than our extracts, and confirmed that leaf extracts showed higher scavenging activity (IC_{50} = 1.41 ± 0.10 mg·mL^{-1}), followed by fruits (1.56 ± 0.12 mg·mL^{-1}) and seeds (2.49 ± 0.21 mg·mL^{-1}), with gallic acid exhibiting an IC_{50} of 0.025 ± 0.002 mg·mL^{-1}. Additionally, El Finou et al. (2024) reported that *C. spinosa* extracts displayed higher antioxidant activity than BHT (IC_{50} = 7.41 vs. 8.31 µg·mL^{-1}). Antioxidant capacity increases with phenolic and flavonoid content, largely determined by the number of aromatic rings and the nature of hydroxyl groups.

RP-HPLC examination recognized 16 phenolic compounds in both extracts. Sinapic acid was the predominant phenolic compound in the methanolic extract, whereas gentisic and benzoic acids were present in very little quantity. Notably, several phenolic compounds were not detected in the aqueous extract. HPLC analysis confirmed that the methanolic extract contained higher phenolic content than the aqueous extract. Aliyazicioglu et al. (2013) also identified gentisic, sinapic, and benzoic acids in the methanolic extract of *C. spinosa L.*, some of which have contributed to the discovery of novel therapeutics. Furthermore, *C. spinosa* L. has been found to have high antioxidant capacity and glucosinolate content (Amer et al., 2023; Yousefi et al., 2025).

In whiteleg shrimp (*L. vannamei*), dietary supplementation with aqueous and methanolic *C. spinosa* L. extracts was tested in this study for effects on growth, immune response, antioxidant activity, immune-related gene expression, and *Vibrio parahaemolyticus* resistance. Supplementation doses of 3.0% kg^{-1} diet methanolic extract substantially impacted growth, antioxidant status, and immunological parameters. The shrimp given the methanolic extract grew better than those fed the aqueous extract. Flavonoids and phenolic glycosides, such as quercetin and kaempferol derivatives, promote feed palatability and growth (Shahrajabian et al., 2021). Methanolic extract contains a higher concentration of phytochemicals compared to aqueous extract. Similarly, El Finou et al. (2024) found that rainbow trout given 0.1 g kg^{-1} methanolic caper extract for 30 days had improved growth and nonspecific immunity. A study by Abdel-Tawwab et al. (2023) demonstrated that feeding African catfish (*Clarias gariepinus*) 12 g kg^{-1} clove basil leaf extract (CBLE) for 12 weeks increased performance and feed utilization. Adeshina et al. (2021) found that feeding Nile tilapia fry Tridax procumbens leaf extract (TPLE) at 6 g kg^{-1} diet increased feed consumption by improving intestinal morphometry. These data suggest that herbal methanolic extracts could serve as aquaculture feed supplements.

The inclusion of *C. spinosa* extracts substantially ($P< 0.05$) enhanced feed utilization and growth rate in *L. vannamei* in comparison with control. Higher supplementation levels, particularly of the methanolic extract, yielded greatest improvements. Nevertheless, there were no obvious changes between shrimp given 2.0 and 3.0% aqueous extract and 1.0% methanolic extract. The highest feed utilization was recorded in shrimp fed MEE 3.0%. According to (Tlili et al., 2011), caper are poor in fat and calories

and high in vitamins and fiber. However, it provides significant quantities of vitamins A, C, and K, modest amounts of vitamin E, and B vitamins (B1, B3, B6, and B9) (Ulukapi et al., 2016). Additionally, it has vital minerals that promote metabolic processes, including calcium, iron, potassium, phosphorus, magnesium, zinc, and manganese. The detected growth boost may be attributed to these nutritional qualities and bioactive compounds (Pakravan et al., 2018). Also, this response may be explained by improved physiological efficiency resulting from enhanced antioxidant capacity and immune regulation. By reducing oxidative stress and lowering the energetic cost of immune maintenance, therefore, dietary energy can be redirected toward somatic growth and protein deposition. Consequently, the marked increase in growth parameters reflects improved energy allocation rather than enhanced nutrient digestion.

Nonspecific immunological measures were considerably elevated ($P< 0.05$) in shrimp given methanolic extracts compared to the control. These results demonstrate that *C. spinosa* extracts effectively enhance immune responses in *L. vannamei*. In shrimp, disease management and immune status are critical to the stability of aquaculture (Al-Musalam et al., 2014). The shrimp immune system comprises cellular and humoral components (Roy et al., 2020). Increased THC following dietary supplementation reflects immune recognition of foreign particles. Hemocytes are central to pathogen recognition and response via phagocytosis, encapsulation, signaling, and nodule formation (Khalil et al., 2023). Hemocyte counts typically decline post-infection due to migration to infected tissues and apoptosis in hematopoietic tissues, both of which are associated with increased mortality (Eissa et al., 2023). Similar declines in hemocyte counts following *V. parahaemolyticus* challenge have been reported (Li et al., 2008). The lower cumulative mortality in shrimp fed 3.0% methanolic extracts aligns with these observations.

SOD and CAT were higher in shrimp fed 3.0% methanolic extracts than in those fed aqueous extracts or the control. On the other hand, malondialdehyde (MDA) values were meaningfully lowest in the 3.0% methanolic extract treatment. In crustaceans, innate immune responses are primary defenses, and hemolymph antioxidants and MDA are useful immune indicators (Soltani et al., 2019). Phenolic compounds in *C. spinosa* extracts may reduce oxidative stress by scavenging free radicals (Annaz et al., 2022). MDA, a marker of lipid peroxidation, indicates free radical-induced damage (Marzouk et al., 2025). The lowest MDA levels in the extract-fed groups corroborate the findings of (Peixoto et al., 2016). Diets supplemented with MEE 3.0% significantly ($P< 0.05$) enhanced hemocyanin (Hc) and Penaeidin−3a (Pen-3a) gene transcription. The innate immune system in crustaceans relies on cells, enzymes, and antimicrobial peptides (Huang et al., 2020).

The significant upregulation of Hc and Pen-3a genes in shrimp fed methanolic *C. spinosa* L. extracts, particularly at the MEE 3.0% level, indicates activation of key components of the innate immune system. Hemocyanin plays a multifunctional role in oxygen transport and immune defense (Shi et al., 2026), while Pen-3a is a potent antimicrobial peptide, suggesting enhanced immunological readiness rather than immune overstimulation (Aweya et al., 2021). The dose−dependent increase in Hc and Pen-3a transcription suggests that methanolic extracts effectively modulate immune gene expression, which may contribute to improved disease resistance observed during the bacterial challenge. This immune enhancement likely reduces subclinical infections and immune−related stress, indirectly supporting better growth performance and overall shrimp health.

Cumulative mortality and relative percent survival following *V. parahaemolyticus* challenge, which has no mortality, occurred in the negative control treatment (injected with saline). Shrimp fed *C. spinosa* extracts exhibited substantially ($P< 0.05$) the lowest mortality in the group fed MEE 3.0%. Similar challenge models have been reported (Yildirim-Aksoy et al., 2022). Post-challenge, bacterial colonization in lymphoid tissues and cellular degradation typically occur by day 7 (Fadel et al., 2025; Van de Braak et al., 2002). High bacterial loads overwhelm shrimp immunity, leading to mortality. Clinical signs of acute hepatopancreatic necrosis disease (AHPND) in challenged shrimp included anorexia, lethargy, erratic swimming, chromatophore expansion, opaque musculature, empty guts, and mortality (Aguilera-Rivera et al., 2019; Fadel et al., 2025; Liu et al., 2021). Extract-treated shrimp exhibited milder, transient signs. Improved growth, feed utilization, survival, and innate immunity were observed in extract-fed groups, leading to lower cumulative mortality and higher relative survival post-challenge. Optimal herbal supplement doses in aquatic animals range from 0.01% to 10% over 15-80 days.

The semi−quantitative scoring complements qualitative pathology (Shackelford et al., 2002). The bacterial challenge with *V. parahaemolyticus* demonstrated a clear protective effect of *C. spinosa* L. extracts, as evidenced by the dose−dependent reduction in gross lesion severity compared with the infected control. Shrimp fed methanolic extracts, particularly at 3.0%, exhibited minimal visible lesions, indicating enhanced resistance to bacterial infection.

The reduced lesion severity in extract−treated groups is consistent with the observed enhancement of immune−related gene expression and suggests improved innate immune defense and tissue protection. This mitigation of pathological symptoms likely reflects better control of bacterial proliferation and lower infection −induced stress, supporting the role of *C. spinosa* L. extracts in improving shrimp health under pathogenic challenge.

Histopathological examination revealed improved hepatopancreatic structure in extract-fed challenged groups compared to control. The hepatopancreas, a multifunctional gland in crustaceans, is involved in digestion, absorption, storage, and hormone secretion (Cao et al., 2023). Control shrimp exhibited typical AHPND lesions, including tubular atrophy, epithelial sloughing, necrosis, and loss of B, R, and F cells, consistently. Milder changes were observed in methanolic extract-fed groups, with less severe tubular damage and signs of recovery. These findings underscore the protective and restorative potential of *C. spinosa* extracts against *V. parahaemolyticus* infection.

5 Conclusion and recommendations

In summary, nutritional supplementation with *Capparis spinosa* methanolic extract 3.0% kg^{-1} of feed significantly improves

growth, survival, feed efficiency, antioxidant capacity, and immunological responses in *Litopenaeus vannamei*. The methanolic extracts exhibited immunoprotective and resistance-promoting properties versus *V. parahaemolyticus*. These results underscore the potential of *C. spinosa* extracts contribute to reducing antibiotic use in aquaculture, enhancing disease control, and promoting ecologically friendly practices. While the current results are promising, further research incorporating escalated dosage gradients of *C. spinosa* methanolic extract is recommended to fully map its effectiveness and maximum potential.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Ethics committee for animal care at the King Salman International University, El-Tor, Egypt. Approval number: KSIU/2026/DA-3. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

HB: Data curation, Formal analysis, Methodology, Writing – original draft. RK: Software, Conceptualization, Writing – review & editing, Methodology, Formal analysis, Supervision, Data curation. HA: Supervision, Writing – review & editing. HK: Writing – review & editing, Supervision, Writing – original draft, Visualization, Formal analysis, Validation, Conceptualization, Investigation, Resources.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2026.1845788/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Phenolic component peak retention durations and peak areas of C. spinosa L. aqueous and methanolic extracts.

SUPPLEMENTARY FIGURE 2
Lesion severity observed in *L. vannamei* shrimp fed on different levels of *C. spinosa L.* extract post-challenge intramuscularly with different densities of *V. parahaemolyticus*. In these groups of patients, symptoms such as melanization, enlarged chromatophores, necrotic regions in uropods, and pale muscle (particularly in the location where the bacteria were injected) were seen, (n=5). Treatments: T1, A basal control diet (- C. *spinosa* - V. *parahaemolyticus*), T2, A basal control diet (- C. *spinosa* +*V. parahaemolyticus*), T3, T4, T5: (+ 1.0, 2.0, and 3.0% of AQE *C. spinosa* +*V. parahaemolyticus*), T6, T7, T8: (+ 1.0, 2.0, and 3.0% of MEE *C. spinosa* +*V. parahaemolyticus*).

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