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EFFECTS OF DIETARY CURCUMIN NANOPARTICLES (C-NPS) ON GROWTH PERFORMANCE, DIGESTIVE ENZYMES ACTIVITIES, ANTIOXIDANT STATUS AND IMMUNITY RESPONSES FOR ENHANCING THE INTESTINAL MUCOSA OF WHITE-LEG SHRIMP (*LITOPENAEUS VANNAMEI*) CHALLENGED BY *FUSARIUM SOLANI*

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Abstract

Currently, the aquaculture industry is challenged with disease outbreaks and pathogen infections originating from intensive aquaculture production. Using phytogenic natural compounds as dietary curcumin nanoparticles that have been extensively applied in aquafeeds to enhance the performance, antioxidant activity, and innate immunity of several fish species, the present study investigates the response of white-leg shrimps (*Litopenaeus vannamei*) challenged with *Fusarium solani* fed on dietary curcumin nanoparticles (C-NPs) on growth, digestive enzymes activity, antioxidant enzymes activity, and their humoral immune responses. White-leg shrimps, *L. vannamei* (3.56±0.02 g) were raised in triplicate groups for 56 days on a diet containing 39% protein and 11% lipid and 5 concentrations of C-NPs (0 as the control, 15, 30, 45, and 60 mg/kg). The pathogen, *F. solani* confronted the shrimps after the feeding trial. The findings showed that the performance parameters FBW, WG, SGR and FCR increased significantly (P≤0.05) by 19.5%, 34.82%, 18.47%, 18.33% and 3.07% with increasing the concentrations of dietary C-NPs. The amount of 45 mg/kg C-NPs in shrimp feed served as an optimum dosage. The control diets had the highest cumulative mortality of white-leg prawns when they were exposed to the pathogen (70.00%), followed by T1 (55.00%) and T2 (45.00%), while T3 and T4 recorded the lowest cumulative mortality rates (35.00%). The intestinal layers (mucosa) of infected shrimps treated by different concentration of C-NPs were significantly improved by 15%. Therefore, the current study recommended using dietary C-NPs to enhance the white-leg shrimp's functionality, digestive and antioxidant enzymes activities and immune system response.

Key words: white-leg shrimp, curcumin nanoparticles, growth performance, digestive enzymes, antioxidants, immunity biomarkers, histology

As a crucial source of food for both animals and people, crustaceans play an essential function in ecological systems (Eissa et al., 2022 a; Okon et al., 2023). Shrimps are considered one of the most consumed crustaceans on the earth. Numerous farmers have entered the shrimp industry as a result of rising global demand for marine shrimp. It is one of the mariculture industry's most profitable enterprises (Khanjani et al., 2016). The Pacific white prawn *Litopenaeus vannamei*, is the most prominent penaeid species that is widely grown and possesses characteristics that are suitable for widespread aquaculture (Khanjani et al., 2016; Chen et al., 2018; Ahmed et al., 2025). Disease outbreaks and the spread of pathogen infections are common due to the proliferation of intensive and stressful fish and shellfish cultivation systems (Eissa et al., 2022 c). *Fusarium solani* is the pathogen causing shrimp black spot disease (BSD). The BSD can attack various tissues in shrimp as gills, bronchial chamber and lungs, causing high mortality of *Litopenaeus vannamei*. *F. solani* causes moulting failure, resulting in mortality of diseased shrimp which can lead to decreased shrimp yields and produced deadly mycotoxins (Figueroa et al., 2018; Moretti et al., 2017; Summerell, 2019). Due to these issues, more chemotherapeutics and antibiotics are being used, which increases the danger of drug resist-

ance, immune system suppression, aquatic ecosystem contamination, and consumer health risks (Kohshahi et al., 2019; Sarkheil et al., 2017). To ensure the health of cultured animals and enhance their growth and immunological response, popular feed additives and medicinal herbs, prebiotics and probiotics have been used as phytogenics and phytochemicals in aquaculture feeds as natural immunostimulants and growth boosters (Alagawany et al., 2021; Moniruzzaman and Min, 2020; Hendam et al., 2023; Moaheda et al., 2023;Abd El-Aziz et al., 2024; Dighiesh et al., 2024).

Curcumin is a significant medicinal herb that contains bioactive compounds as hydrophobic and polyphenolic substance that act as antimicrobial, antioxidant, immune modulating, and gastro-protective (Eissa et al., 2022 d; 2023 b; 2024 a, b; Jastaniah et al., 2024).

The nutritionist tends to use nanoparticle curcumin which has superior aqueous medium dispersion and absorption compared to bulk curcumin (Ghalandarlaki et al., 2014; Moniruzzaman and Min, 2020). The beneficial effects of curcumin are well documented, e.g. antibacterial, anti-cancer, anti-inflammatory, antioxidant, chemoprotective, gastroprotective, growth promoting, hepatoprotective, immunostimulants, neuroprotective effects and antioxidant candidate in diets of various fish species and shrimp. Several investigations demonstrated the benefits of using nano-scale feed supplements to enhance the welfare and performance of Pacific white shrimp *Penaeus vannamei,* Nile tilapia fingerlings, European sea bass (*Dicentrarchus labrax* L.), red tilapia (*Oreochromis* sp.) and green tiger shrimp (Moghadam et al., 2021; Abdel-Tawwab et al., 2021; Eissa et al., 2023 b). The efficiency and well-being condition of prawns may be enhanced by dietary curcumin nanoparticles (C-NPs). The present study examined the effects of food fortification with C-NPs on *L. vannamei* development rate, gastrointestinal enzyme levels, level of antioxidants, and immunological state.

Material and methods

Curcumin nanoparticles synthesis

The materials used for curcumin nanoparticle synthesis were purchased from Chemajet Company (Cairo, Egypt) and dichloromethane acquired from Elgomhoreya pharmaceutical company (Cairo, Egypt). The solvent method previously described by Abdel-Tawwab et al. (2021), with some minor modifications, was used to synthesize the nanocurcumin (NC) particles. Briefly, a syringe pump comprising antisolvent was used to manufacture NC utilizing dichloromethane as an organic solvent (Kakran et al., 2012). Firstly, the original curcumin solution was prepared in dichloromethane (5 mg/mL), positioned in a syringe (20 mL), and injected at a proportion of 10 mL/min into the deionized water (antisolvent), and stirred (1000 g) for two hours. At that point, the vacuum-dried manufactured nanoparticles were cleaned. A Zeta sizer (Malvern Instruments, Zeta Potential Analyser, Malvern, UK) was utilized to assess the NC measurement (Figure 1). Moreover, the electron microscope (EM) was also applied to assess the size of synthesized NC and its distribution (particle size 10–50 nm). The particle's average diameter of curcumin nanoparticle was 11.1 nm (Figure 1).

Experimental protocol

L. vannamei juveniles were acquired from a private farm in Damietta and then delivered to the containers in bags of plastic containing water and oxygen. In cages, fifteen hapas ($1 \times 1 \times 1$ m^3) were separated into five duplicate groups (T1 to T5). Each hapa was supplied with 30 juveniles of the white leg prawn *L. vannamei* (IBW, 3.49±0.04 g). The sinking pellet diet was fed to the shrimp fingerlings twice per day. Shrimp were fed six days per week at a feed rate of 5% of the total biomass. All groups of shrimp fingerlings were measured every two weeks, and the quantity of feed was changed in response to variations in body weight over the course of the experiment. The experiment was conducted for eight weeks and five *L. vannamei* for each hapa had been gathered. They were evaluated for their chemical structure using the techniques outlined by AOAC (1995).

Diet preparation

Table 1 shows the ingredients used for diets formulation and approximate chemical composition of a control diet with 38.78% CP. Various levels of C-NPs (0, 15, 30, 45, and 65 mg/kg diet) were added to the control diet to formulate tested diets. The C-NPs were then evenly sprayed with 100 mL of distilled water while suspended, added to the diet's components, thoroughly mixed for 30 minutes, and then formed into 2 mm-diameter pellets. To be used later, the prepared meals were placed in plastic bags and kept at 4°C. The AOAC procedures (1995) were used to analyse the meals to determine their chemical composition (Table 1) as outlined by Eissa et al. (2023 a, b).

Shrimp were gathered from each hapa at the end of the experiment and they were numbered and measured in groups. The feed utilisation indices and shrimp growth parameters were computed as follows:

WG (g) = Final weight (g) - Initial weight (g).

ADG (g/fish/day) = (Final weight (g) - Initial weight (g))/Days of feeding trial.

SGR (%) = [(ln final weight − ln initial weight) no. of days] ×100.

SR (%)= (Final number of fish/Initial number of fish) × 100.

FI (g/fish) = the amounts of feed consumed throughout the investigational period/fish (g).

FCR: (Total feed consumption/Weight gain of fish).

Protein efficiency ratio = Weight gain (g)/Protein ingested (g)

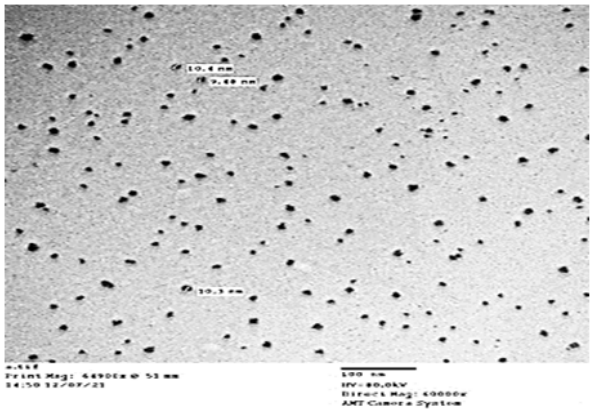

Figure 1. Nanocurcumin (NC) characterization by electron microscopy

Table 1. Chemical and compositional evaluation of experimental diets

Ingredients	g/kg diet	Proximate chemical analysis g/kg diet	
Wheat flour	120.0	Dry matter	90.64
Shrimp meal	250.0	Moisture	9.35
Rice bran	70.0	Crude protein (N × 6.25)	38.79
Soybean meal	150.0	Crude fat	10.90
Fish meal	300.0	Crude fibre	1.74
Fish oil	60.0	Ash	6.15
CMC[4]	10.0	Carbohydrate (NFE)	32.975
Vit. & Min. Mix[1]	20.0	Gross energy kcal/100g[3]	459.467
Min. mix[2]	20.0		
	1000		

[1]Vitamin premix (per kg of premix): 2.5 g thiamine; 2.5 g riboflavin; 2.0 g pyridoxine; 100.0 g inositol; 0.3 g biotin; 100.0 g pantothenic acid; 0.75 g folic acid; 2.5 g para-aminobenzoic acid; 200.0 g choline; 10.0 g nicotinic acid; 0.005 g cyanocobalamine; 20.1 g a-tocopherol acetate; 2.0 g menadione; 100,000 IU retinol palmitate; 500,000 IU cholecalciferol.

[2]Mineral premix (g/kg of premix): $CaHPO_4.2H_2O$: 727.2; $MgCO_4.7H_2O$: 127.5; KCl: 50.0; NaCl: 60.0; $FeC_6H_5O_7.3H_2O$: 25.0; $ZnCO_3$: 5.5; $MnCl_2.4H_2O$: 2.5; Cu (OAc) $2.H_2O$: 0.785; $CoCl_3.6H_2O$: 0.477; $CaIO_3.6H_2O$: 0.295; $CrCl_3.6H_2O$: 0.128; $AlCl_3.6H_2O$: 0.54; Na_2SeO_3: 0.03.

[3]Gross energy (GE) was measured from NRC (2011) as 16.7, 37.4, and 16.7 kJ/g for carbohydrates, lipid and protein, respectively.

[4]Carboxymethyl cellulose.

Assessment of water quality

During the experimental period, the water variables were assessed daily using A Hanna HI-9147 automated probe following the method of Rice (2012). The water temperature, salinity, pH, dissolved oxygen, and NH_4^+ were 26.87±0.12°C, 2.03±0.03, ppt, 7.80±0.00, 5.93±0.02 mg/L and 0.34±0.00, respectively.

Proximate body composition

Five shrimp from each hapa were collected to measure body chemical composition, including dry matter (DM), crude lipid (CL), crude protein (CP), and ash. All these variables were measured using standard methods (AOAC, 1995). The crude lipid and crude protein were determined by the Soxhlet technique and by nitrogen analysis after acid digestion using the Kjeldahl manner, respectively. Lipid was measured using Soxhlet instrument. The DM was assessed by drying at 105°C until the weight of samples remained constant, while the percentage of ash was assessed after 13 hours of drying at 550°C in an electric oven.

Immune parameters analysis

Phagocytosis activity

Hemolymph (200 mL) was collected from the base of the third walking leg and mixed with 800 mL of sterile anticoagulant. Collected shrimp hemocytes were rinsed with shrimp saline and the viable cell number adjusted to $1*10^6$ cells/mL. The cell suspension (200 mL) was inoculated into a cover slip. After 20 min, the cell suspension was removed and rinsed with shrimp saline three times. Heat-killed yeast preparation (2 mL) was added and incubated for 2 h. Next, the heat-killed yeast preparation was removed and the cell suspension rinsed with shrimp saline five times to reach the concentration of $5*10^8$ cells/mL and fixed with 100% methanol. Then, the cover slip was stained with Giemsa stain and mounted with Permount slide mounting fluid. Two hundred hemocytes were counted for each sample. Based on Chotigeat et al. (2004), a phagocytic action (PA) test was performed. PA and PI were computed using the earlier described formula (Watanuki et al., 2009).

Phagocytic activity was expressed as Percentage phagocytosis = phagocytic hemocyte/total hemocyte * 100.

Total hemocyte count (THC)

Hemolymph (100 µL) of individual shrimp was withdrawn from the pleopod base of the second abdominal segment with a sterile 1 mL syringe (25 G × 13 mm needle). Before hemolymph extraction, the syringe was loaded with a precooled (4°C) solution (10% EDTA, Na_2) used as an anticoagulant. The hemolymph with anticoagulant solution was diluted in 150 µL of formaldehyde (4%) and then 20 µL were placed on a hemocytometer (Neubauer) to determine the total hemocyte count (THC) using an optical microscope (Olympus, DP72).

Lysozyme (Lys) activity

Lysozyme activity was measured by using a lysozyme detection kit (Sigma-Aldrich, Cat. no. LY0100) according to the manufacturer's instruction. The results were defined by the lysis of the *Micrococcus lysodeikticus* cells. The reactions were conducted at a temperature of 25°C and the absorbance was measured at a wavelength of 450 nm in the ultraviolet/visible spectrophotometer (Perkin Elmer, Lambda XLS, USA):

$$Lysozyme\ activity = (\Delta A_{450}/min\ Test - \Delta A_{450}/min\ Blank)\ (d_f) / 0.001 \times 0.03$$

Digestive enzymes activity

Trypsin activity was measured as follows: 40 µl of the enzyme extract was diluted in 160 µl of 50 mM Tris-HCl pH 8.2 buffer containing 20 mM $CaCl_2$. Next,

20 µl aliquots of this new solution were incubated with 100 µl of substrate solution containing 1 mM BApNA, 50 mM Tris-HCl pH 8.2 and 20 mM $CaCl_2$ at 30°C using a 96-well acrylic microplate. The reaction rate was expressed as specific activity, defined as nmol of 4-nitroaniline produced per minute (U) per mg protein (mU^{-1} mg protein). The molar extinction coefficient of the 4-nitroaniline used to perform the calculations was 8800 M/cm.

Chymotrypsin activity was measured as follows: 20 µl of the enzyme extract was diluted in 160 µl of 100 mM Tris-HCl pH 7.8 buffer containing 25 mM $CaCl_2$. Next, 20 µl aliquots of this new solution were incubated with 140 µl of substrate solution containing 1.13 mM BTEE, 100 mM Tris-HCl pH 7.8 and 25 mM $CaCl_2$ at 30°C using 96-well UV treatment acrylic microplate (Corning Incorporated, Corning, New York, USA). Activity was measured by reading the absorbance at 256 nm every 20 s for 20 min. The reaction rate was expressed as specific activity, defined as nmol of tyrosine produced per minute (U) per mg of protein (U/mg of protein). The molar extinction coefficient used to perform the calculations was 964 M.cm.

Protease activity was determined using 0.5% hemoglobin as a substrate at pH 2, as described elsewhere (Montoya et al., 2010). One unit of protease activity was defined as 1 mg of tyrosine released per minute and mg of protein.

Antioxidant activity

The fish serum levels of malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were assessed by colorimetric methods based on using diagnostic reagent kits (Biodiagnostic Co, Egypt). MDA as an indicator of lipid peroxidation was measured according to the method described by Mendes et al. (2009). The activities of CAT, SOD, and GPx were detected following the procedures outlined by McCord and Fridovich (1969), Morris and Albright (1981), and Nakano et al. (1992), respectively.

Challenge with fungus

The challenge with fungus followed the protocol described by Eissa et al. (2022 d, 2023 b). Shrimp were collected, pooled and distributed at a density of 10 per 50-L tank, with three duplicates of each treatment. The third abdominal segment of the animals received an intramuscular injection containing 0.1 ml and 5×10^4 conidia/ml. The animals were fed the proper meals for an extra 15 days while abnormal symptoms and prawn mortalities were monitored. The feeding experiment and the challenge trials both used the same salinity, dissolved oxygen, and water temperature. Excrement and unused feed were emptied every two days. To isolate and identify the fungus, prawn gill and muscle tissue samples from recently killed shrimps were taken and placed on PDA plates.

Histological study

The livers and the intestines of the control and the treated fish were fixed in 10% neutral buffered 10% formalin, and the samples were then dehydrated in ascending grades of ethyl alcohol, cleared in xylol, embedded in molten paraplast at 56°C and cut at 5 µ on rotator microtome. The paraffin sections were stained with hematoxylin and eosin (Drury and Wallington, 1980). Histopathological studies were undertaken through light microscopy and photomicrographs were made.

Results

Water quality

During the experimental period, DO was about 6.60±0.03 mg/L, ammonia nitrogen level was less than 1.22±0.01 mg/L, toxic ammonia was about 0.13±0.00 The temperature was 27.13±0.07°C, salinity was 30.50±0.04 g/L, and pH was 8.16±0.01.

Growth performance and survival

The growth efficiency and feed utilisation of *L. vannamei* are shown in Table 2 in terms of performance. In comparison to the control group, fingerlings were found to be considerably greater in T4 and T3 than in T2 and T1. The specified growth rate (SGR) was kept constant in line with the growth results. When compared to the control, feed intake was considerably ($P<0.05$) greater in T4, T3, T2, and T1, but not statistically ($P>0.05$) different from T1. The feed conversion ratios continued a similar pattern. The shrimp groups evaluated in this study, from control to T4, did not show any changes in survival rate (%) that were statistically significant.

Table 2. White-leg prawn (*L. vannamei*) growth performance, feed utilisation, and survival rate following 56-day feeding experiments

Parameters	Control	T1	T2	T3	T4
Initial weight (g)	3.56±0.02	3.47±0.04	3.47±0.06	3.45±0.04	3.51±0.04
Final weight (g)	12.32±0.25 a	13.05±0.18 b	14.41±0.16 c	14.98±0.17 d	15.32±0.09 d
Weight gain (g)	8.76±0.23 a	9.58±0.14 b	10.94±0.17 c	11.52±0.19 d	11.81±0.13 d
SGR (%/day)	2.22±0.03 a	2.36±0.01 b	2.54±0.04 c	2.62±0.02 cd	2.63±0.03 d
Feed intake (g/animal)	15.76±0.12 a	16.07±0.17 a	16.65±0.11 b	17.04±0.16 bc	17.37±0.03 c
Feed conversion ratio	1.80±0.03 d	1.68±0.01 c	1.52±0.02 b	1.48±0.01 b	1.47±0.02 b
Survival rate (%)	72.22±4.01	75.56±1.11	78.89±1.11	78.89±1.11	74.44±1.11

Significant changes are indicated by superscript letters in the same row ($P<0.05$).

Table 3. Proximate body composition of white-leg shrimp after 56-day feeding trials

Parameter	Control	T1	T2	T3	T4
Crude protein	16.16±0.09 a	16.65±0.18 b	16.70±0.04 b	17.06±0.11 c	16.96±0.03 bc
Crude lipid	1.90±0.04 b	1.82±0.01 a	1.78±0.03 a	1.74±0.02 a	1.77±0.01 a
Ash	3.05±0.03 a	3.12±0.02 b	3.14±0.01 b	3.14±0.02 b	3.17±0.02 b

Significant changes are indicated by superscript letters in the same row (P<0.05).

Table 4. Status of digestive enzymes activities (U/mg protein, mean ±SD, P<0.05) of white-leg shrimp (*L. vannamei*) fed on dietary supplements for 56 days

Parameter	Control	T1	T2	T3	T4
Chymotrypsin	0.10±0.01 a	0.14±0.02 ab	0.13±0.01 b	0.15±0.03 b	0.15±0.02 b
Trypsin	0.12±0.02 a	0.13±0.03 a	0.15±0.03 ab	0.17±0.02 b	0.17±0.01 b
Protease	1.01±0.01 a	1.12±0.01 a	1.19±0.01 b	1.22±0.01 b	1.22±0.01 b
Lipase	0.02±0.00 a	0.04±0.01 b	0.05±0.01 bc	0.06±0.02 c	0.06±0.01 c
Amylase	0.91±0.02 a	1.14±0.00 b	1.16±0.01 b	1.19±0.01 c	1.20±0.00 c

Significant changes are indicated by superscript letters in the same row (P<0.05).

Table 5. Status of antioxidant activity (U/mg) and lipid peroxidation (U/mg) biomarkers of white-leg shrimp fed on supplements for 56 days. Significant changes are indicated by superscript letters in the same row (P<0.05)

Parameter	Control	T1	T2	T3	T4
Superoxide dismutase (SOD)	36.23±0.06 a	41.12±0.52 b	41.96±0.73 b	45.51±0.41 c	46.75±0.15 c
Catalase (CAT)	3.55±0.05 a	4.36±0.02 b	4.40±0.03 b	4.51±0.03 c	4.65±0.05 c
Glutathione peroxidase (GPx)	57.68±0.56 a	64.48±0.55 b	67.49±0.43 c	69.85±0.50 d	69.58±0.64 d
Malondialdehyde (MDA)	7.32±.29 c	6.59±0.07 b	6.24±0.11 ab	5.81±0.01 a	5.87±0.05 a

Significant changes are indicated by superscript letters in the same row (P<0.05).

Table 6. Status of immune response of white-leg shrimp (*L. vannamei*) fed on dietary nanocurcumin supplements for 56 days

Parameter	Control	T1	T2	T3	T4
THC ($\times 10^7$ cells/mL)	25.62±0.49 a	32.58±1.07 b	33.27±0.24 bc	34.63±0.43 c	35.07±0.37 c
RB activity (U/μg protein)	0.33±0.02 a	0.54±0.02 b	0.58±0.01 bc	0.59±0.01 c	0.60±0.01 c
Lys activity (U/μg protein)	13.94±0.10 a	16.30±0.24 b	18.12±0.11 c	19.28±0.44 d	19.97±0.21 d
PO activity (O.D. at 490 nm)	1.14±0.03 a	1.49±0.03 b	1.75±0.04 c	2.05±0.03 d	2.19±0.04 e
Phagocytic activity (%)	25.44±0.35 a	26.53±0.16 b	28.42±0.41 c	29.96±0.21 d	29.99±0.11 d
Phagocytic index	4.01±0.18 a	4.15±0.05 a	5.11±0.23 b	5.33±0.07 b	5.55±0.07 b

Significant changes are indicated by superscript letters in the same row (P<0.05).

Proximate composition of shrimp body

Table 3 shows the *P. vannamei* fingerlings' compositions. Following the shrimp groups of T4, T2, T1, and the control, the T3 shrimp group had the greatest crude protein content (P<0.05). When compared to the shrimp groups treated with T1, T2, T3, and T4 treatments, the crude lipid in the control group was found to be considerably greater (P<0.05). The control group's shrimp had considerably (P<0.05) less ash than the shrimp in the other C-NPs-treated shrimp groups, although not by a dramatic (P<0.05) amount.

Digestive enzymes activities

The activity of chymotrypsin was dramatically (Table 4, P<0.05) superior in the shrimp groups at T4, T3, T2 and T1 treatments compared to the control whereas trypsin was almost similar to the trend of chymotrypsin, there was no difference among the shrimp groups at T1 and control treatments. There was a significant increase of protease in the shrimp groups at T4, T3 and T2 treatments relative to the control and T1. Lipase was dramatically elevated in the shrimp groups at T4 and T3 treatments followed by T1 and control but increased non significantly compared to T2 shrimp groups. The digestive enzyme activity for amylase was significantly improved in the shrimp groups of T4 and T3 treatments followed by the shrimp groups at T2 and T1 treatments relative to the control.

Antioxidant activities

The antioxidant activities were influenced by the addition of different concentration of dietary C-NPs. Table 5 exhibited that all the antioxidant activities in the shrimps treated at different concentration of C-NPs were dramatically (*P*<0.05) higher compared to the shrimp groups at control, except the malondialdehyde (MDA) activities which was dramatically (P<0.05) higher at the basal diet.

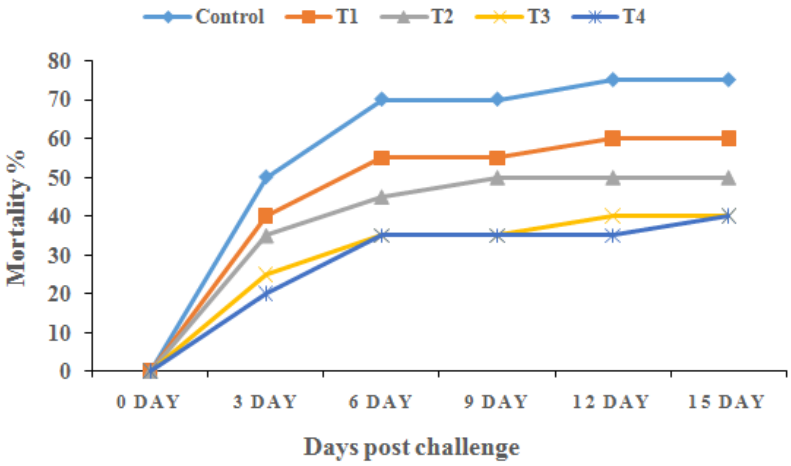

Figure 2. Cumulative mortality (%) status of shrimps during post-challenge

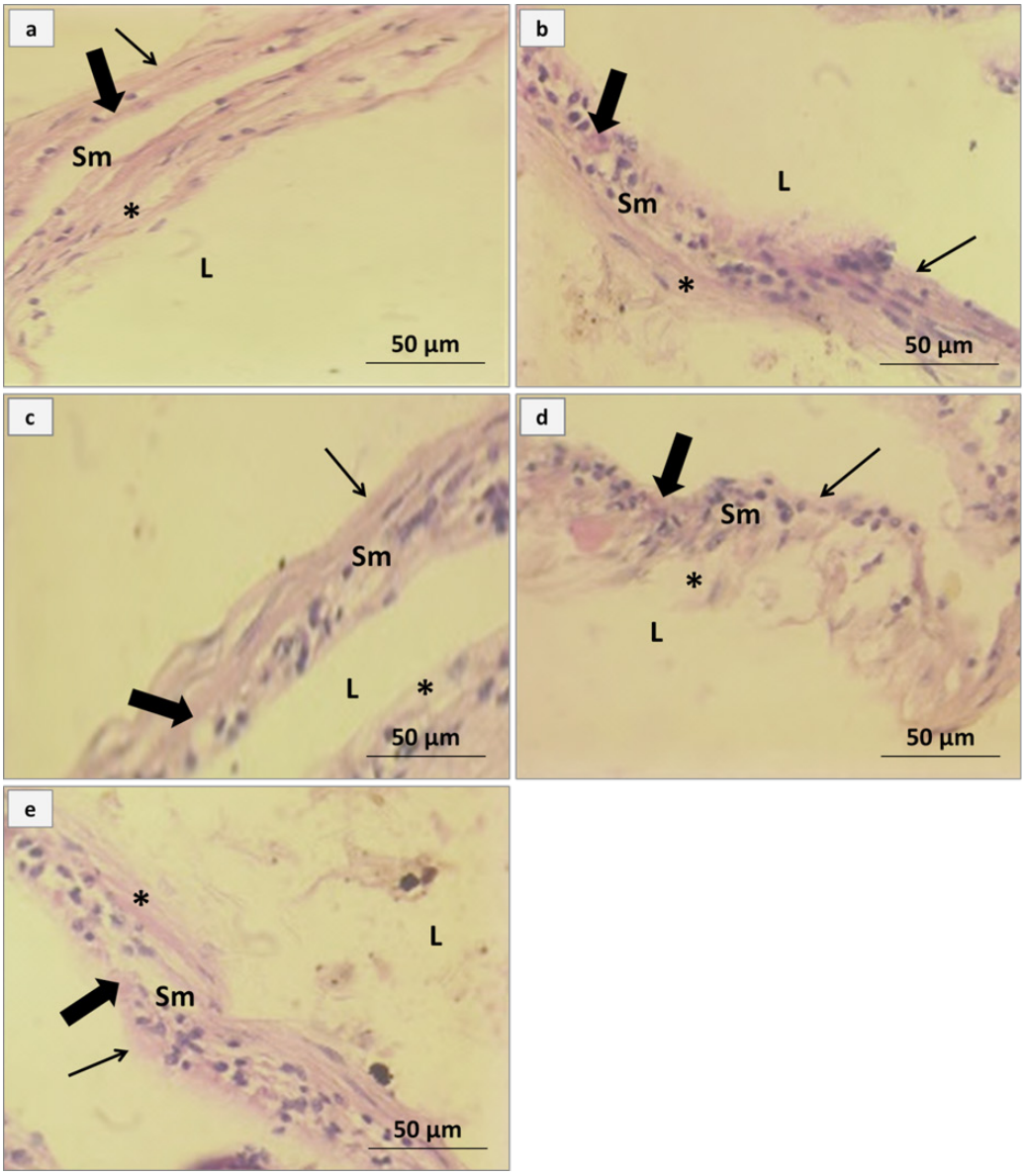

Figure 3. Photomicrograph of white-leg shrimp (*L. vannamei*) intestine tissue samples for five experimental groups after challenge with *Fusarium solani*. a: control group (T0), b: T1, c: T2, d: T3, and e: T4. L: lumen, asterisks: mucosa layer, Sm: submucosa layer, thick arrows: muscularis layer, and thin arrows: serosa layer. [H&E, Scale bar = 50 µm]

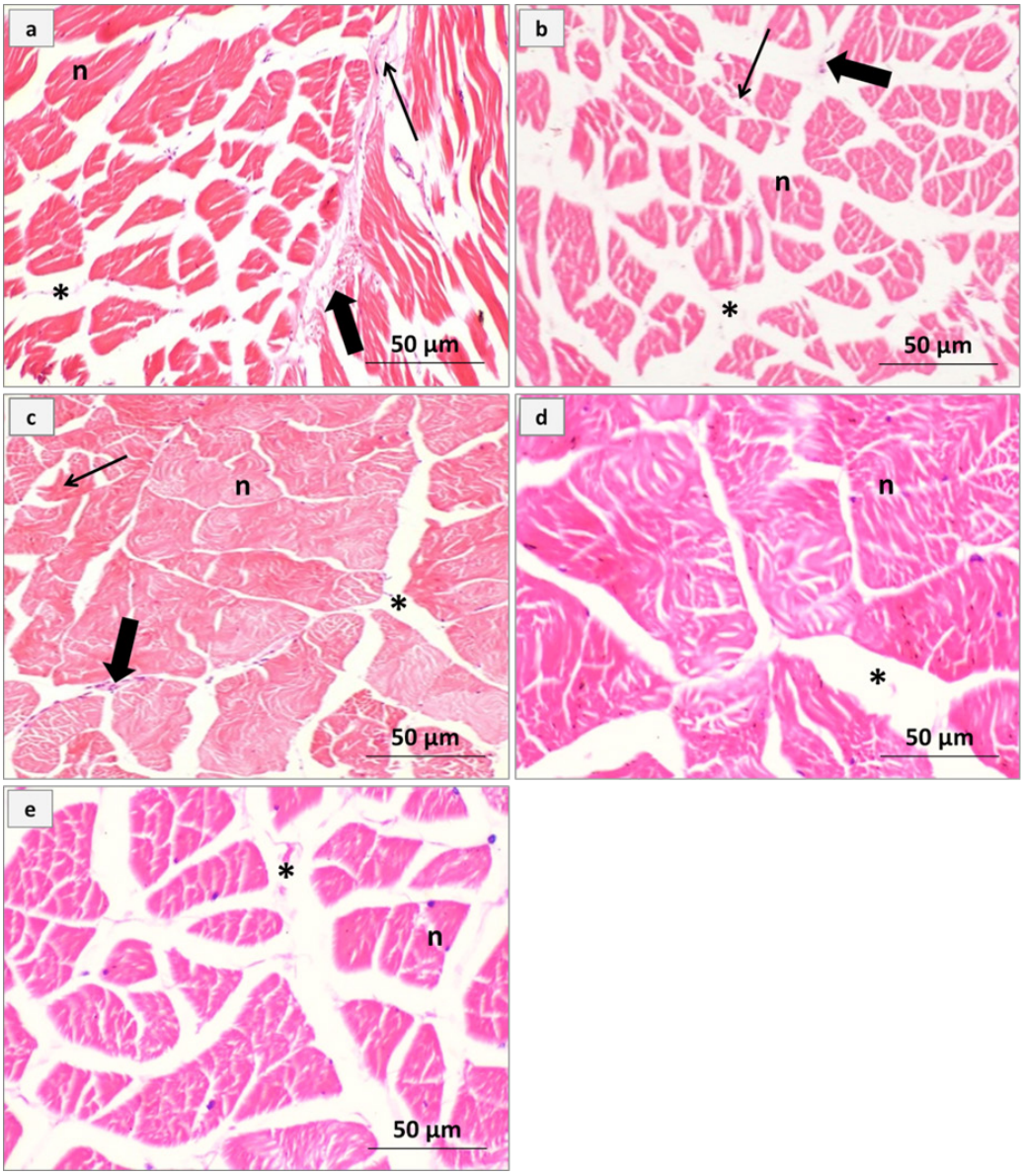

Figure 4. Photomicrograph of white-leg shrimp (*L. vannamei*) muscle tissue samples for five experimental groups after challenge with *Fusarium solani*. a: control group (T0), b: T1, c: T2, d: T3, and e: T4. n: nucleus, asterisks: loose connective tissue, thin arrows: myofibres clumping, thick arrows: immunocytes. [H&E, Scale bar = 50 µm]

Innate immunity parameters

The higher concentration of C-NPs led to the higher innate immunity of studied shrimps (Table 6). The total hemocytes count ($\times 10^7$ cells/mL) and respiratory burst activity (U/µg protein) were significantly increased in shrimp groups at T4 and T3 treatments followed by T1, T2 and the control. Similar trends were maintained in the lysozyme (Lys) activity, the phenol oxide activity. There were no differences in the phagocytic actions and index between the shrimp groups at T3 and T4 treatments, these are dramatically ($P<0.05$) higher relative to the shrimp groups T2, T1 and the control.

Challenge study with *Fusarium solani*

Dense aerial white to cream mycelium, an abundance of floccose and reverse brown colouring on PDA, and an abundance of ellipsoidal, fusiform or kidney-shaped microconidia after 2–3 days in fresh isolates were the morphological characteristics of *F. solani*. Macroconidia were established thick-walled, with 3–4 septa, straight ahead, and primarily parallel-sided after 4–7 days. The apical cell's blunt tip was curved. Additionally, as compared to shrimps that appeared to be healthy, the severely afflicted ones had gills that were a dark shade of black. The diseased shrimps had collapsed, atrophic, and necrotic lesions on their gill lamellae.

Shrimp fed with feeds containing nanocurcumin enriched diets were more resilient to *Fusarium solani* infection (Figure 2) as contrasted with shrimps fed with the basal diet, which recorded the maximal death rate of shrimp.

Histological examination

The shrimp intestine tissue samples were improved (Figure 3) of the intestinal layers, especially mucosa layer in shrimp groups at T4, T3 and T2 treatments compared to the control (T0). Figure 4 exhibited that the muscle tissue samples had enhanced muscle fibres in shrimp groups at T3 treatment compared to the shrimp groups at T0, T1, T2 and T4 treatments.

Discussion

The current study was conducted to test the implications of curcumin nanoparticles (C-NPs) on development, gastrointestinal health, and oxidative enzyme activity in white-leg prawns (*L. vannamei*) challenged with *Fusarium solani*. The current study has shown that, in comparison to control diets without C-NPs, the introduction of C-NPs considerably improved final body weight, weight gain, specific growth rate and feed conversion ratio incidence of white-leg prawns (*L. vannamei*). The current findings are in line with those of Yonar et al. (2019) who discovered that rainbow trout provided diets enriched with 20 g/kg curcumin showed significantly improved growth, FCR, and survival rate. In addition, Mahmoud et al. (2017) observed that Nile tilapia growth and feed efficiency indicators were enhanced by inclusion of curcumin at two levels (50 or 100 mg/kg diet). Moreover, feeding Nile tilapia curcumin at dosages of 5, 10, or 20 g/kg may improve growth parameters and feed efficiency (El-Barbary, 2018; Cui et al., 2017). Furthermore, Jiang et al. (2016) found that carp fed diets containing 5 mg/kg of curcumin demonstrated improved growth markers and digestive enzyme activity. Also, carp provided diets containing 5 mg/kg of curcumin showed better growth indicators and digestive enzyme function, as reported by Jiang et al. (2016). Similar findings were made by Mohamed et al. (2020) who discovered that inclusion of curcumin (200 mg/kg) in the diets of Nile tilapia fish quadratically increased growth metrics and rate of survival. According to Eissa et al. (2022 d) nanocurcumin can be regarded as a helpful nutritional supplement for Nile tilapia at 25–40 mg/kg inclusion level, and at optimal levels of 50–60 mg/kg diet for red tilapia (Eissa et al., 2023 b). The current findings might be explained by a number of ways: i) curcumin alters the ecology of gut bacteria by way of the positive bacteria (Jiang et al., 2016); (ii) increases the activities of digestive enzymes and their absorption (Yonar et al., 2019); (iii) modifies the anatomy of the stomach and intestines; and (iv) improves and stimulates shrimp appetite (Jiang et al., 2016); (v) enhances fish gut nutrition absorption and eliminates pathogen microorganisms (Jiang et al., 2016); (vi) the outcome of prebiotic metabolic processes is a short chain of fatty acids that are acquired by epithelial cells and can be implemented as a source of power for helping in the assimilation of nutrients (Yonar et al., 2019); and (vii) C-NPs are an excellent supply of prebiotic for the formation of lactic acid bacteria (Yonar et al., 2019).

Inclusion of NC to the diet greatly boosted the protein and ash content of the shrimp's body while only slightly lowering body lipid levels. Recent studies revealed that red tilapia muscles' ash and protein contents were dramatically raised by long-term curcumin feeding (Eissa et al., 2023 b). Moreover, Cao et al. (2018) found that inclusion of curcumin in gibel carp diet increased the ash and protein content of the whole body while lowering the lipid quantities. The present results could be attributed to the following scenario: i) the regulation of the intestinal microbiota, which enhanced the efficient nutrient utilization (Jiang et al., 2016); ii) another explanation would be the beneficial effects of curcumin on the activity of fish digestive enzymes including trypsin, lipase, and amylase (Mahmoud et al., 2017); and iii) negatively charged particles, such as nanocurcumin, slow down the adsorption rate of serum proteins, resulting in longer circulation half-lives than positively charged particles (Rastiannasab et al., 2016). This could explain why fish fed diets containing nanocurcumin had a higher crude protein content than fish fed free-curcumin diets.

The current study showed that, in comparison to control diets, the digestive enzyme activity was enhanced in shrimp given diets supplemented with C-NPs/kg diet. The current findings are in line with those reported by Jiang et al. (2016) who discovered that feeding a diet enriched with 1 or 5 g of curcumin kg^{-1} feed increased the activity of lipase and protease enzymes in crucian carp. Additionally, *Seriola dumerili*'s intestines' lipase and trypsin capabilities were dramatically boosted by including 0.01% curcumin in the food (Li et al., 2020). Moreover, red and Nile tilapia (Eissa et al., 2022 d) require optimal inclusion of approximately 50–60 mg/kg food and 25–40 mg/kg diet, respectively, of nanocurcumin to increase digestive enzymes (Eissa et al., 2023 b). In addition, Qi-Cun et al. (2007) found a positive correlation between high nutrient digestive enzymes activities and inclusion of curcumin in the diet. The present study showed that the growth-promoting impact of curcumin might be attributed to its ability to enhance digestive enzyme activity in the hepatopancreas and intestine (Abd El-Hakim et al., 2020)

In terms of oxidative enzyme actions, SOD, GPx, and CAT levels were increased while MDA levels decreased in shrimp given diets enriched with either 45 or 60 mg C-NPs/kg. The current findings are consistent with those of Abdel-Tawwab et al. (2022) who discovered that adding C-NPs considerably boosted SOD, CAT, and GPx activities in addition to dramatically reduced MDA levels. Similar to this, Jiang et al. (2016) reported that crucian carp (*Carassius auratus*) given dietary curcumin (5 mg/kg) demonstrated increased antioxidant enzyme activity. Furthermore, Moghadam et al. (2021) discovered that *L. vannamei* fed with various doses of nanocurcumin showed substantial rises in SOD and CAT activity together with reduced MDA levels. In addition, Mahmoud et al. (2017), Yonar (2018) and Yonar et al. (2019) found that adding curcumin to the diet

dramatically increased the antioxidant activity of Nile tilapia, common carp, and rainbow trout (Mahmoud et al., 2017; Yonar, 2018; Yonar et al., 2019). The results of the current study could be explained by a number of different scenarios, including: (i) the antioxidant activity of C-NPs, which could scavenge free radicals and stimulate antioxidant parameters (Manju et al., 2012; Xu et al., 2018); (ii) curcumin's antioxidant properties, which involve the activation of the nuclear factor erythroid 2 (Nrf2) signalling pathway, which is involved in free radical scavenging; iii) according to Bishayee et al. (2011) curcumin's high concentration of polyphenols, which function as hydrogen or electron donors and have the potential to stabilise unpaired electrons and stop Fenton processes, may be the cause of these effects; iv) curcumin contains polyphenolic chemicals that block the nitrosation process, increase the SOD and GPx activities, and scavenge reactive oxygen species to reduce oxidative damage (Moskaug et al., 2005).

The immunological response of white-leg prawns fed diets enriched with either 45 or 60 mg C-NPs/kg was enhanced as measured by THC, RB activity, Lys activity, PO activity, and phagocytic index. The current findings are in line with those reported by Abdel-Tawwab et al. (2022) and Eissa et al. (2023 a), who discovered a clear rise in TP, ALB, GLO, LYZ, and total Ig levels in fish fed with C-NPs. Similar research revealed that feeding *L. vannamei* NMC-enriched meals resulted in greater levels of LYZ, TP, and ALB than the control group, according to Moghadam et al. (2021). The current study might be related to several scenarios, including: i) curcumin is a potential source of prebiotics that could enhance the conversion of inorganic nitrogen in water to microbial nitrogen according to Abdel-Tawwab et al. (2022); ii) curcumin and *L. plantarum* work together to create a favourable intestinal environment; iii) administration of curcumin may boost natural probiotics in water reaching fish's stomach and induce a stronger immune response (Moghadam et al., 2021; Eissa et al., 2022 d); iv) curcumin administration increases disease resistance and non-specific immune responses (Moghadam et al., 2021); v) curcumin, a widely used component of turmeric, is known to help prevent a variety of inflammatory illnesses, which in turn enhances growth and feed effectiveness (Moghadam et al., 2021); and vi) curcumin is a component of turmeric extract (TE) acting as a possible prebiotic and anti-inflammatory chemical (Moghadam et al., 2021).

The cumulative mortality rate (35%) of shrimp fed a meal supplemented with 45 or 60 mg C-NPs/kg was lower than that of the control group (70%) in comparison. These findings suggested that the development and immunological responses of Nile tilapia exposed to *Fusarium solani* might be improved by the addition of adequate dietary C-NPs, particularly at doses of 45 or 60 mg C-NP per kg diet. The current findings are in line with those of Yuan et al. (2014) who discovered that juvenile Wuchang bream (*Megalobrama amblycephala*) fed diets enriched with 30 and 60 mg/kg had a reduced cumulative death rate relative to the control group.

The present findings could be attributed to the function of C-NPs to operate as an anti-inflammatory, antioxidant, and immunological stimulant may be the cause of its ability to lower the cumulative mortality rate (Eissa et al., 2022 d, 2023 b). These findings suggested that proper dietary C-NP supplementation, particularly at doses of 45–60 mg/kg diet, might increase shrimp immune responses, growth, and resistance to *Fusarium solani*. The present results demonstrated that shrimp given diets supplemented with C-NPs/kg diet showed an improvement in the intestinal and muscular tissue samples of the treatment groups (T3, T4, and T2, respectively) compared to the diets used as controls. The current findings are in line with Abdel-Tawwab et al. (2022) and Eissa et al. (2022 d, 2023 b), who demonstrated that the addition of nanocurcumin to fed diets improved the architectural composition of aquatic animals' tissue, highlighting the positive effect of nutrition in terms of disease resistance. Earlier studies have also shown the importance of diet in the occurrence and severity of various fish illnesses through the immune system's regulation (Blazer, 1992).

Conclusions

Shrimp's growth performance, feed utilisation, survival rate, histological implications, and disease resistance towards *Fusarium solani* infection were all enhanced through the inclusion of nutritional NCs at multiple concentrations in the shrimp diet. These findings recommended inclusion level of nanocurcumin ranging from 45 to 60 mg/kg diet, and nanocurcumin might be regarded a helpful nutritional supplement for prawns.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of fish were followed by the authors and the Scientific Research Ethics of the National Institute of Oceanography and Fisheries 'NIOF', Egypt – Certificate number (NIOF- AQ3-1-23-R-036).

Consent for publication

All authors have reviewed and approved the manuscript for publication.

Conflict of interest

The authors declare no competing interests.

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Data availability statement

All data regarding this study are presented in the paper.

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Author contribution

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