

RESEARCH ARTICLE

Growth, Digestive Enzyme Activities, Antioxidant Properties, and Intestinal Histology of Nile Tilapia (*Oreochromis niloticus*) Fed Diets Supplemented With Garlic Nanoparticles

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ABSTRACT

This work may be the first of its kind to assess the effects of varying inclusion levels of garlic nanoparticles (NPsG) as feed additives on growth and physiological indices in Nile tilapia (*Oreochromis niloticus*). Garlic contains an organosulfur compound called allicin that can promote growth and strengthen the immune system by stimulating appetite and the intestinal flora, thus enhancing energy utilization. Nanotechnology provides new tools in aquaculture nutrition, including increasing the ability of fish to absorb nutrients rapidly and the effectiveness of feed additives by enhancing their bioavailability compared to their bulk form. Five concentrations of NPsG (0, 150, 300, 600, or 1200 mg/kg) were evaluated by their addition to a basal diet (30% crude protein). 225 juveniles with an initial weight of 3.92 ± 0.42 (SE) were randomly divided into 15 cages of polyethylene. The cage had dimensions of length: width: height (100:40:60 cm) and was continuously aerated using a 2 HP air blower with air stones. After 42 days, feeding results revealed that NPsG supplementation had significant effects ($p < 0.05$) on improving the performance, physiological indicators, and histomorphometry of the intestine in comparison with the control. Fish that were fed a dietary supplement of 600 mg/kg of NPsG exhibited the highest specific growth rate (2.80%/day), along with elevated levels of intestinal enzymes lipase, amylase, and proteases (212.40, 15.00, and 318.60 U/g of intestine, respectively) and the lowest malondialdehyde level (81 nmol/g of liver tissue). This group was followed by fish fed a diet of 300 mg/kg of NPsG, which did not significantly differ from those fed 600 mg/kg of NPsG in superoxide dismutase, total antioxidant capacity, and histomorphometry of intestine villi. Finally, the complete dose–response recommendation (benefit at 300–600 mg/kg and detriment at 1200).

1 | Introduction

The Nile tilapia, *Oreochromis niloticus*, is a prominent species in aquaculture worldwide, making a large contribution to economic output (El-Sayed 2006). However, tilapia farming in intensive systems is accompanied by several challenges, such as reduced growth rates due to stress and deterioration of water quality,

increased susceptibility to diseases, and widespread antibiotic abuse (Fadel et al. 2024). This emphasizes how important it is to use sustainable techniques to increase tilapia farming's resilience and output (Cabello et al. 2013). Therefore, immunostimulants are increasingly being used in aquaculture to boost disease resistance in finfish and increase the activation of nonspecific defensive mechanisms. However, there are some disadvantages

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to using antibiotics or other chemotherapeutics, including the development of resistant bacteria and adverse effects on human health and the environment (El-Dakar et al. 2023; Abdel-Tawwab et al. 2022). Furthermore, certain feed types exhibit reduced efficacy or bioavailability, inconsistent absorption, and nutrient loss during processing and storage (Filipe et al. 2023; Nunes et al. 2022). Fish diets enriched with nanoparticles (NPs: sizes from 1 to 100 nm) offer a viable alternative approach to supporting sustainable aquaculture, with unique chemical and physical characteristics that can be used to get around some of the restrictions outlined above (Thangapandiyar et al. 2020). Because of their large surface area-to-volume ratio, NPs can interact with biological systems at the molecular level, facilitating precise targeting and nutrient uptake. As a result, nutritional absorption and bioavailability are improved (Mekuye et al. 2023). These NPs' high surface area-to-volume ratio also makes it possible to load and encapsulate vital nutrients and medicinal substances effectively. Additionally, NPs can be created in special shapes such as rods or discs, which often exhibit longer circulation durations (Lawson et al. 2024). NPs can be engineered into various shapes, such as rod-shaped or disk-shaped designs, often exhibiting longer circulation times. These shapes can improve stability, reduce clearance by the immune system, and facilitate better targeting and uptake by specific cells or tissues (Lawson et al. 2024), leading to more effective delivery of nutrients and increased feeding efficiency (Adel et al. 2024). These advantages boost performance, reduce waste and costs, and improve production quality and yield (Chen and Yada 2011). Additionally, compared to the control, it is hypothesized that the garlic NP (NPG) formulation will demonstrate superior performance by increasing dispersion and absorption in aqueous media (El Basuini et al. 2022).

It is commonly known that fish mucosal immunity can be enhanced by medicinal herbs and their extracts (Doan et al. 2020). Fish skin mucus, which is the first layer of the innate immune system, is made up of various innate immune components, including complement, lysozyme, protease, alkaline phosphatase, lectin, and catalase (Caipang and Lazado 2015). Utilizing natural bioactive substances as feed additives and taking advantage of their growth-promoting, immunostimulatory, and antimicrobial properties is a crucial tactic for sustainable operations (Abdel-Aziz et al. 2023). One interesting approach is garlic (*Allium sativum*), which has positive effects on blood pressure, cholesterol, and blood sugar, as well as a natural antibacterial due to its allicin content (Olusola et al. 2013). Garlic supplements can improve immunity, growth, appetite, and flesh quality in aquaculture (Lee and Gao 2012). Furthermore, Abdel-Tawwab et al. (2022) reported that garlic powder at 1% and 2% as feed additives could increase digestibility and feed efficiency of crayfish, and at 3% achieved the highest protein efficiency ratio (PER) and feed intake, along with improved villus structure and favorable histological features in the intestine and liver of hybrid grouper *Epinephelus fuscoguttatus* × *E. lanceolatus* (Zidan et al. 2025). This study examines herb-enhanced fish feed with NPs, emphasizing both its potential advantages and disadvantages. It focuses on how different NPs might enhance aquaculture's sustainability, health, and growth. It aimed to evaluate the effects of different concentrations of NPGs as supplemental feeds on growth, digestive enzymes, antioxidant capacity, intestinal histology, and body composition in Nile tilapia.

2 | Materials and Methods

2.1 | Declaration of Ethics

The research protocol and experimental practices for this work received approval from two ethics committees. The research adhered to the guidelines set by the Arish University Research Ethics Review Committee in Egypt, which approved the protocol under code ARU050. Additionally, the Scientific Research Ethics Committee of Suez Canal University, Faculty of Agriculture, based in Ismailia, Egypt, also approved the protocol with code 21/2024. Every effort was made to minimize pain during the experimental procedures. The authors affirm that the study complies with the ARRIVE guidelines.

2.2 | Site of Works

The Aquaculture Unit in Desert Regions (AUDR), Faculty of Marine Aquaculture and Fisheries, Arish University, Arish, North Sinai, Egypt, provided the facilities for this study.

2.3 | Preparation NPGs

NPGs were prepared using a green synthesis method adapted from Mostafa (2025). The steps were as follows:

1. Initial Extraction

Peel and slice fresh garlic cloves and rest (aeration): expose garlic at room temperature for 10–15 min to form the illicit.

- Mix 50 g of garlic with 100 mL distilled water (or 70% ethanol) in a high-speed blender for 5 min.
- Whatman No. 1 filter paper was used to filter the extract in order to remove any suspended particles.

2. Size Reduction to Nanoscale (Top-Down Approach)

- To achieve ~60 nm particles, ultrasonically (probe sonicator) is recommended.

– Ultrasonication Protocol:

- Dilute the crude extract 1:10 with distilled water.
- Use a probe sonicator (400–600 W, 20 kHz frequency).
- Place the sample in an ice-water bath to prevent overheating.
- Program: 10 cycles, each cycle: 5 min on, 1 min off (at 50% amplitude).

– Postsonication:

- Centrifuge the sonicated mixture at 3000 rpm for 10 min to remove larger particles.
- Collect the supernatant, which contains the NPGs.

3. Drying and Stabilization

- To obtain a dry nanopowder (as in your analysis report):

Lyophilization (freeze-drying): Add a cryoprotectant such as trehalose (2% w/v) to prevent aggregation. Or rotary evaporation at low temperature (below 40 °C).

4. Narrowing Particle Size Distribution (PSD, Reducing SPAN)

- To achieve a very narrow distribution
 - Nanofiltration using a 50 nm membrane.
 - Or, ultracentrifugation at 20,000 rpm for 30 min to select particles within the 50–70 nm range

5. Characterization Confirmation

PSD was measured using a laser particle analyzer (Better size 2600 particle analyzer; Dandong Better Size Scientific Ltd., Dandong, China) equipped with an automatic laser centering function.

Synthetic NP characterization: The morphology and PSD. The produced NPs fell within the nanoscale range (<100 nm), according to the PSD analysis, as shown in Figure 1. The differential distribution (red line) and cumulative distribution (blue line) are both displayed on the graph. Excellent control over particle size and dispersion is demonstrated by the fact that 100% of the particles are between 0.050 and 0.100 µm, which is crucial for applications involving NPsG. The D10, D50, and D90 values further confirm the narrow particle-size distribution (Chen et al. 2021; Sharma et al. 2023).

Moreover, the shape and stability of the synthesized NPsG were assessed by Zeta sizer (Malvern Instruments, Zeta Potential Analyzer, Malvern, UK), and an electron microscope was used to assess the size of synthesized NPsG according to (Baruah et al. 2023; Williams et al. 2009).

2.4 | Fish, Rearing Conditions, and Experimental Design

A total of 225 Nile tilapia juveniles, weighing an initial average of 3.92 g ± 0.42 (SE) at the beginning, were used. Fifteen plastic cages were housed in a greenhouse with dimensions of (length × width × height) 100 cm × 40 cm × 60 cm (with a water volume of 80 L).

Fish were randomly divided into cages at a rate of 15 fish/cage. Underground water (with a salinity of 3–4) was used, with a photoperiod of 12 h L:12 h D and a water change rate of 20% daily. Air stones were used to regularly aerate each cage using a 2 HP (150–200 L/min) air blower. Five experimental meals including varying doses of NPsG supplementation (0, 150, 300, 600, or 1200 mg/kg diet) were examined for 42 days.

2.5 | Diets and Feeding

The control was a basal diet with ≈30% crude protein (0 mg/kg NPsG) that was compared to four different levels of NPsG as follows: NPsG150, NPsG300, NPsG600, and NPsG1200 at doses of 150, 300, 600, or 1200 mg/kg, respectively (Table 1). NPsG was added on top of the basal diet (supplemental), ensuring that the diets remain essentially isonitrogenous/isocaloric. The basal diet was formulated with fishmeal, soybean meal, yellow corn, wheat bran, starch, vitamins, minerals, and plant oil (Table 1). All ingredients were milled and weighed according to the formula and mixed with a prescribed dose of NPsG supplementation for each treatment (starch was used as a carrier or filler for the tested doses of NPsG to increase homogeneity, then added to the mixture of ingredients), and then a little water was added to this mixture until it became dough, and then it was pelleted by a meat mincer. After being sun-dried, the pellets were kept at 4 °C until they were needed. Fish were fed twice daily at 9 a.m. and 4 p.m. at a rate of 3% of their body weight. A sample of each treatment was weighed every 15 days to modify the feeding rate.

2.6 | Water Quality

Temperature, dissolved oxygen, pH, and ammonia levels were measured daily and periodically. MP and MQ Multi-Parameter Water Quality Sensors (Model MP47 or MQ65) were used to determine the temperature (°C), dissolved oxygen (DO, mg/L), and pH. Total ammonia nitrogen (mg/L) was estimated weekly by chemical methods using a spectrophotometer model (LKB Bichrom UV–visible spectrophotometer) at a wavelength of 630 nm (APHA 2005). Means of water parameters were 24.40 ± 0.36 °C, 5.48 ± 0.06 mg/L, 8.20 ± 0.07, and 0.076 ± 0.002 mg/L for temperature, dissolved oxygen, pH, and total ammonia nitrogen,

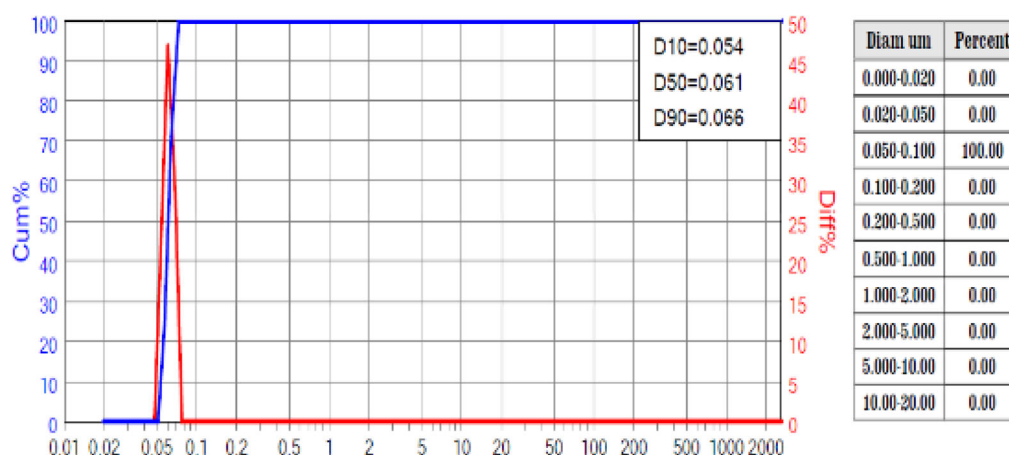


FIGURE 1 | Distribution of nano garlic practical size (µm). From analysis of Bettersizer 2600 (Wet).

TABLE 1 | Ingredients of basal diet, levels of NPsG and a proximate chemical composition on basis dry matter.

Ingredients, g/1000 g	Control	Treatments			
		NPsG 150	NPsG 300	NPsG 600	NPsG1200
Fish meal	155	155	155	155	155
Soybean meal	360	360	360	360	360
Yellow corn	340	340	340	340	340
Wheat bran	100	100	100	100	100
Starch	20	20	20	20	20
Soy oil	20	20	20	20	20
Vitamin and mineral ^a	5	5	5	5	5
Total	1000	1000	1000	1000	1000
Nanoparticles of garlic, mg/kg	—	150	300	600	1200
Chemical composition of a basal diet without additives on basis dry matter, %					
Crude protein			29.84		
Ether extract			7.07		
Crude fiber			0.87		
Ash			10.56		
Nitrogen free extract ^b			45.03		
Gross energy, kcal/g			4.22		

^aVitamins and minerals mixture, each 1 Kg of mixture content: 62.5 m I.U. vit A, 25 m I.U. vit D3, 25 g vit E, 1.75 g vit K, 0.5 g vit. B1, 2.75 g vit. B2, 1.25 g vit. B6, 10 mg vit. B12, 20 g niacin, 500 mg folic acid, 50 mg biotin, 37 g zinc, 22 g iron, 31 g manganese, 2.5 g copper, 50 mg cobalt, 113 mg selenium, 650 mg iodine.

^bNitrogen-free extract, determined by difference = 100 – (CP% + fat% + CF% + ash%).

and were within the acceptable range according to [El-Sayed \(2006\)](#), and salinity was in the optimum range for growth, as reported by [Dawood et al. \(2023\)](#). Nile tilapia showed the possibility of growth with normal health status in brackish water (5–10 ppt).

2.7 | Calculations

$$\text{Total weight gain (WG, g)} = \text{Final body weight (W}_f\text{)} \\ - \text{Initial body weight (W}_i\text{)}$$

$$\text{Average daily gain (ADG, g)} = \frac{\text{Total weight gain TG(g)}}{\text{days}}$$

$$\text{Specific growth rate (SGR, \%/day)} = [(\ln W_f - \ln W_i) / \text{days}] \times 100$$

$$\text{Survival rate (SR, \%)} = (\text{No. of fish at the end} / \\ \text{No. of fish at the beginning of trial period}) \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{Feed intake(g)/fish}}{\text{WG(g)}}$$

$$\text{PER: protein efficiency ratio} = \text{WG, g/protein intake, g}$$

2.8 | Sampling Collecting

A random five fish of each replicate ($n = 15$ per treatment) were euthanized by a deep dose anesthetized with MS-222 (0.25 g L^{-1} trichaine methane sulfonate; Sigma–Aldrich–[Dawood et al. 2022](#)), then euthanized by decapitation. Liver and whole digestive tract

samples were aseptically excised from ten fish and immediately immersed in liquid nitrogen and stored at -80°C until analyzing the antioxidant activity and digestive enzymes. Besides, some specimens of the intestines were collected according to standardized necropsy protocol ([Meyers 2009](#)) for the histological study. The remaining five were frozen for analyzing the body chemical composition.

2.9 | Antioxidant Activity and Digestive Enzymes

Superoxide dismutase (SOD; catalog No. SD2521), total antioxidant capacity (TAC; Catalog No. TA 2513), and malondialdehyde (MDA; catalog No. MD2529) were evaluated spectrophotometrically using Bio-diagnostic reagent kits, Dokki, Giza, Egypt. Amylase activity was analyzed by the starch-hydrolysis method described by [Bernfeld \(1955\)](#). Lipase activity was determined using the method delineated by [Furne et al. \(2005\)](#). Also, [Drapeau \(1976\)](#) reported that the casein digestion method (Protease-MBS1601766) was used to measure the protease level.

2.10 | Histopathological Examinations

Each group's intestinal tissue samples were immediately preserved in 10% neutral buffered formalin for 24 h and then dehydrated in graded ethanol (70%–100%) series. Subsequently, the samples were infiltrated in xylene, embedded in paraffin, and then sectioned using a rotary microtome at $5 \mu\text{m}$ thick, stained with hematoxylin and eosin dyes, and examined under a light microscope to identify any pathological changes ([Bancroft and Layton 2019](#)).

The images were first spatially calibrated using ImageJ (version 1.54) to correct for the magnification introduced by the microscope objective lens and the digital camera sensor. The spatial scale was set by a standard stage micrometre, which allowed all morphometric measurements to be expressed in micrometres (μm). The quantitative assessment of the morphology of [insert tissue, e.g., intestinal/duodenal] was made on five randomly selected, well-oriented, nonserial histological sections from each experimental group. In each chosen area, three complete longitudinally sectioned villus regions were chosen and measured. The following morphometric parameters were quantified for each villus using the straight line and freehand line tools in ImageJ: villus length (tip of villus to villus-crypt junction) and villus width (at widest point/mid-point) of each villus. The average value of the five (5) villi measured in each section was determined (Wilson et al. 2018).

2.11 | Fish Body Composition

The AOAC (2005) methodologies were used to determine the approximate chemical components of meals and whole-body fish at the end of the trial. After oven drying at 105 °C until the dry weight was constant, the moisture content was measured. Petroleum ether extraction in a Soxhlet apparatus for 16 h was used to determine the total lipid content, while the micro-Kjeldahl apparatus was used to evaluate the crude protein content. Ash content was calculated from the weight loss after incineration of samples in a muffle furnace at 550 °C to constant weight.

2.12 | Statistical Analysis

Shapiro–Wilk test and Levene’s test were used to check data normality and confirm that experimental groups had equal data spread; hence, the results were analyzed through one-way analysis of variance (ANOVA), and means were compared by Duncan’s Multiple Range test ($p < 0.05$). Using Software (SPSS version 23).

3 | Results

3.1 | Growth, FCR, and SR, %

Results in Table 2 display that treatments differed significantly ($p < 0.05$) in final weight (W_f), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and PER. Fish fed 600 mg/kg diet of NPsG (NPsG600) had the best values of these indices (Figure 2A,B), followed by NPsG300, which did not differ from NPsG150. The lowest performance was recorded by NPsG1200 and the control group. Besides, SR (%) did not change significantly, wherein groups of NPsG (150, 300, and 600) did not record a mortality percentage compared to the NPsG1200 and the control group.

3.2 | Digestive Enzymes of Intestinal Tract

Figure 3A–C demonstrated notable variations ($p < 0.05$) in lipase activity levels between treatments. When compared to the other groups, NPsG600 had the greatest lipase and protease levels, followed by NPsG150. Amylase did not vary significantly; however, NPsG600 had higher amylase activity than NPsG300 and NPsG150, while NPsG1200 and the control had the lowest amylase activity.

3.3 | Antioxidant Assays

Antioxidant activities in liver tissues showed significant differences ($p < 0.05$) among the groups, as presented in Figure 4A–C. NPsG600 and NPsG300 had the highest levels of SOD and TAC, followed by NPsG150, control, and NPsG1200, respectively. However, MDA was the opposite; the lowest MDA was recorded in NPsG600, followed by NPsG300.

3.4 | Histological Investigation

The histological analysis of the intestines from each group in the study was conducted to assess the impact of NPsG on villus

TABLE 2 | Biological and nutritional indices of Nile tilapia fed supplementary diet of NPsG for 42 days.

Items	Groups					<i>p</i> value	PSE
	Control	NPsG150	NPsG300	NPsG600	NPsG1200		
W_i , g	3.96	3.98	3.90	3.91	3.91	0.390	0.01
W_f , g	10.71 ^b	12.29 ^{ab}	12.15 ^{ab}	12.71 ^a	11.30 ^b	0.046	0.27
WG, g	6.75 ^b	8.31 ^{ab}	8.25 ^{ab}	8.80 ^a	7.37 ^b	0.045	0.27
ADG, g	0.160	0.196	0.198	0.210	0.170	0.060	0.01
SGR, %/days	2.36 ^b	2.68 ^{ab}	2.70 ^a	2.80 ^a	2.51 ^b	0.040	0.06
SR%	93.30	100	100	100	93.30	0.390	1.56
FI, g/fish	11.14	12.53	12.36	12.48	10.83	0.190	0.23
FCR	1.66 ^a	1.51 ^{ab}	1.52 ^{ab}	1.34 ^b	1.56 ^{ab}	0.010	0.30
PER	2.02 ^b	2.21 ^{ab}	2.22 ^{ab}	2.45 ^a	2.27 ^a	0.040	0.21

Note: Means with different superscripts letters (a, b, and c) significantly differ at $p < 0.05$.

Abbreviations: ADG, average daily gain; FCR, feed conversion ratio; FI, feed intake; PER, protein efficiency ratio; PSE, pooled standard error; SGR, specific growth rate; SR, survival rate; W_f , final weight, WG, weight gain; W_i , initial weight.

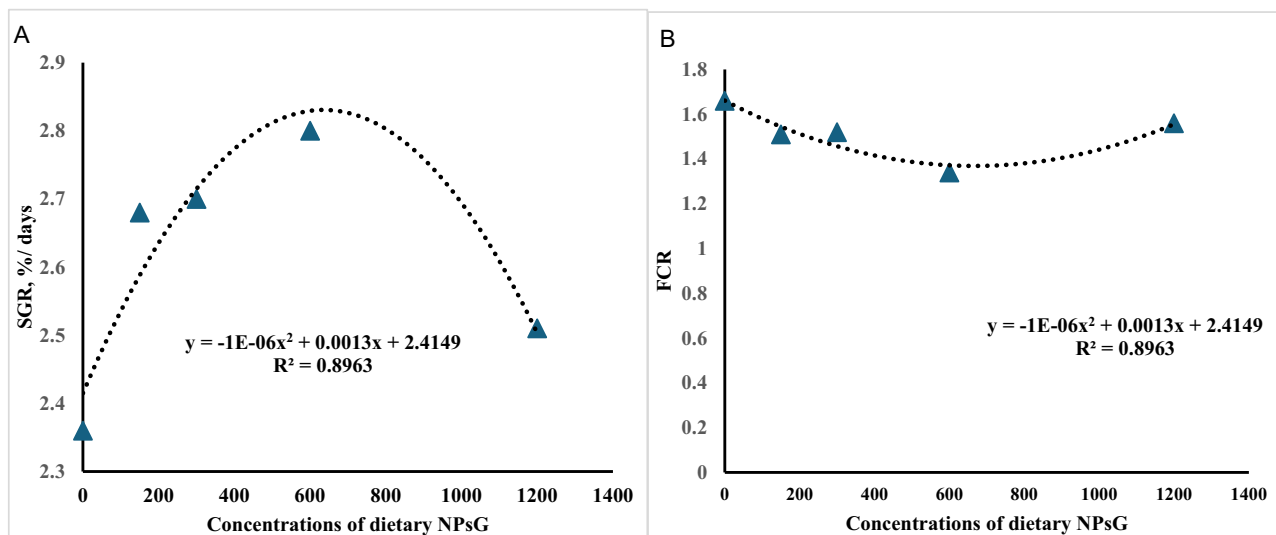


FIGURE 2 | The relationship of biological performance (SGR and FCR) and different doses of garlic as supplanted feed of Nile tilapia for 42 days.

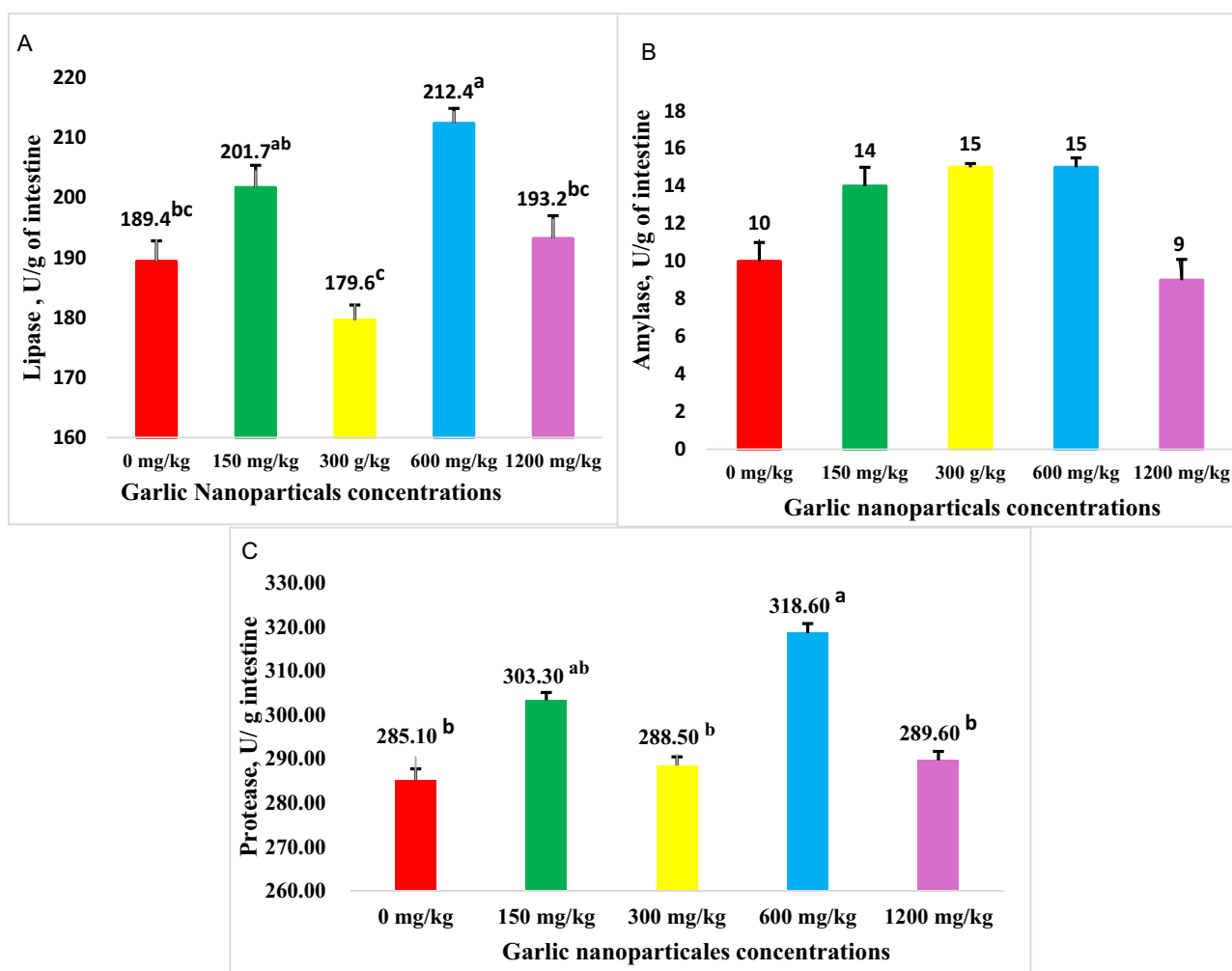


FIGURE 3 | (A–C) Lipase, U/g intestine; amylase, U/g intestine; and protease, U/g intestine of Nile tilapia fed different doses nanoparticles of garlic as supplanted feed for 42 days. Means followed by standard error bars with different superscripts (a, b, and c) significantly differ at $p < 0.05$.

condition. The control group, which was not given NPsG, exhibited a normal mucosal layer arrangement and a typical appearance of the lamina propria supporting the villus epithelial cells. Goblet cells containing transparent mucous droplets were clearly

visible between enterocytes, and both blood vessels and lacteal vessels were observed in the villus core (Figure 5A). Besides, this group had lower villi length and width than the treated groups (NPsG150, NPsG300, and NPsG600), as shown in Figure 6A,B.

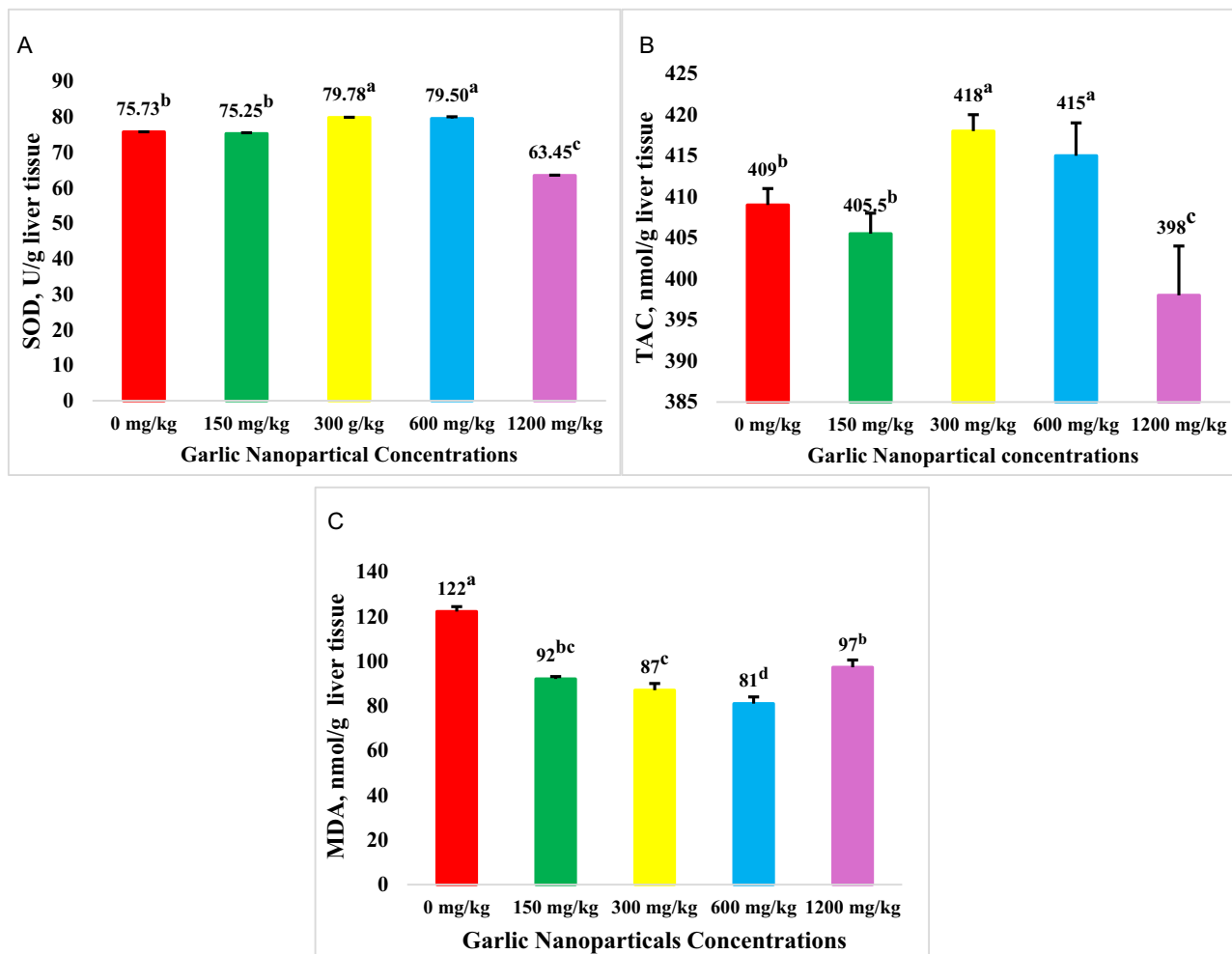


FIGURE 4 | (A–C) Superoxide dismutase (SOD, U/g liver tissue), total antioxidant capacity (TAC, nmol/g liver tissue), and malondialdehyde (MDA, nmol/g liver tissue) of Nile tilapia fed different doses nanoparticles of garlic as supplanted feed for 42 days. Means followed by standard error bars with different superscripts (a, b, and c) significantly differ at $p < 0.05$.

The group fed 150 mg/kg of NPsG showed a normal mucosal layer arrangement and an increased lamina propria, supporting the epithelial cells of the villi. Goblet cells were also observed between enterocytes. Also, blood vessels, lacteal vessels, and some immune cells were observed in the histological section of this group (Figure 5B). In addition, this group showed an increase in villi length (1.72 mm) and width (0.853 mm); however, there was atrophy of some enterocytes in a large number of villi in this group. As illustrated in Figure 6A,B.

The groups that were provided with a diet containing an additional 300 and 600 mg/kg of NPsG, respectively, demonstrated a notably positive influence of these concentrations on villous structure and villous length (1.58 and 1.71 mm) and villous width (0.828 and 0.794 mm), as shown in Figure 6A,B compared to the control group and the remaining group. In these groups, the morphology of epithelial cells appears to be superior to that of the control group and the group of NPsG1200. The mucosal layer is arranged normally, with goblet cells containing transparent mucous droplets interspersed between enterocytes. The lamina propria, which provides support to the epithelial cells, maintains a normal appearance. Blood vessels, lacteal vessels, and immune cells are visible at the center of the villi (Figure 5C,D, respectively).

The fish group that administered a diet containing 1200 mg/kg of NPsG demonstrated an adverse effect of this concentration of NPsG on the normal structure of the villi, as observed in Figure 5E). The histological analysis of this group indicated atrophy in the epithelial cells, enterocytes, and the disappearance of goblet cells, all of which have a negative impact on the absorption process in this group. Moreover, there was an abnormal appearance of blood vessels and lacteal vessels in the core of the villi compared to the other groups (Figure 5E).

3.5 | Whole Body Composition

Data in Table 3 showed insignificant differences in all body composition compounds (protein, fat, and ash) of fish under the different treatments, however, there are merely numerical trends showing that fish of NPsG600 had the highest protein and the lowest lipid content.

4 | Discussions

According to the results shown, the performance and physiological wellness of the fish improved with a supplemented diet of

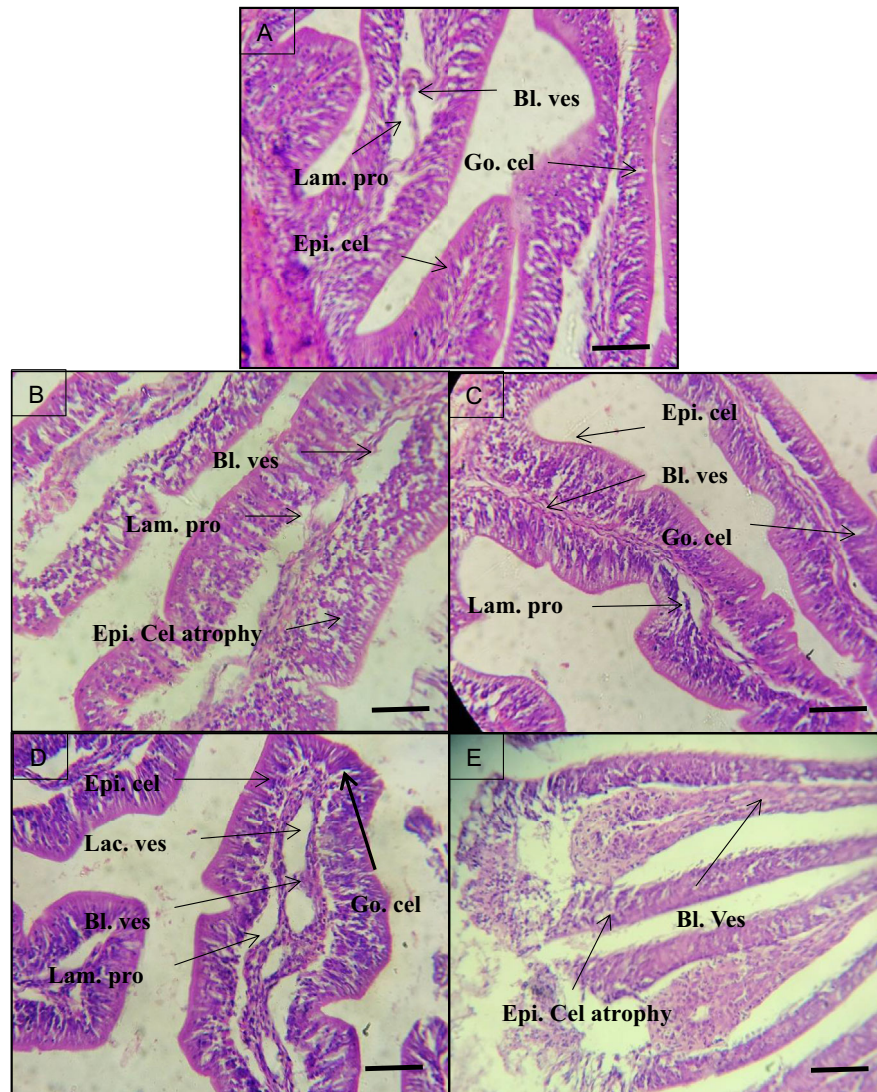


FIGURE 5 | (A–E) Histological sections of intestine (A) show the control group, (B) intestine of the group that fed on a diet with 150 mg/kg of NPsG, (C) show the intestine of the group that its feed contains 300 mg/kg of NPsG, (D) show the intestine sections of group that fed on a diet with supplement of 600 mg/kg of NPsG, and (E) illustrate the intestine of the group that fed on a diet with addition of 1200 mg/kg of NPsG. Abbreviations: Bl. ves: blood Vessel; Epi. Cel: epithelial cells; Go. cel: goblet cell; Lac. ves: lacteal vessel; Lam.pro: lamina propria. Scale bar = 50 µm.

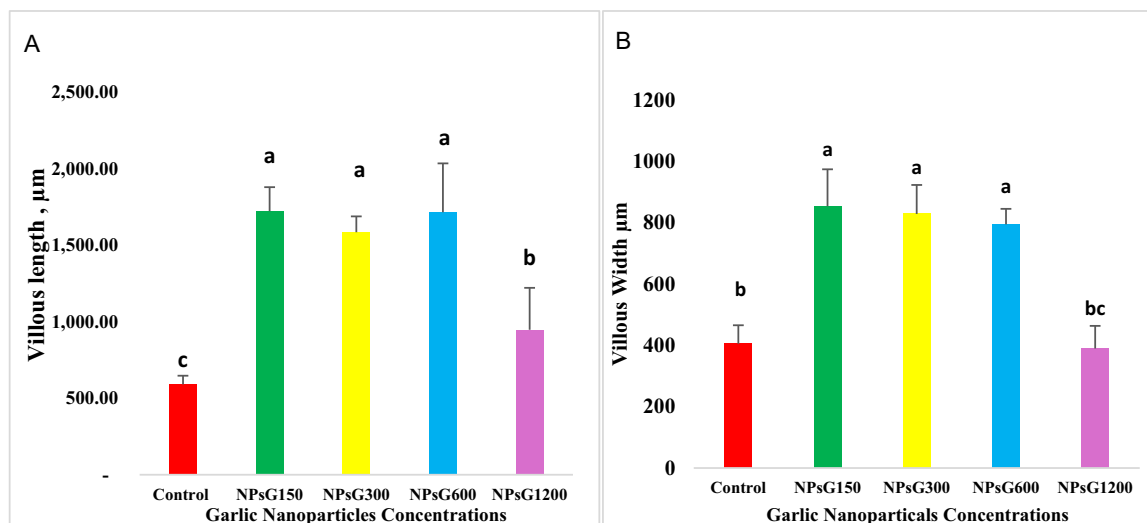


FIGURE 6 | (A,B) Villous length, µm and Villous width, µm of Nile tilapia intestine that was fed dietary nanoparticles of garlic after 42 days. Means followed by standard error bars with different superscripts (a, b, and c) significantly differ at $p < 0.05$.

TABLE 3 | Whole fish body composition of Nile tilapia fed dietary NPsG after 42 days. (On basis dry matter).

Treat.	Groups					p value	PSE
	Control	NPsG150	NPsG300	NPsG600	NPsG1200		
Dry matter, %	25.97	25.53	25.91	24.99	25.40	1.00	1.180
Protein, %	66.9	66.5	66.2	68.7	66.15	0.74	0.680
Fat, %	19.09	19.59	17.16	16.97	19.58	0.07	0.407
Ash, %	13.87	13.87	16.42	13.92	14.34	0.78	0.650

Abbreviation: PSE, pooled standard error.

nano garlic compared to control groups fed diets of 300 and 600 g/kg of NPsG. It has been demonstrated that medicinal plants increase immunity, growth, and the activity of digestive enzymes, which enhances aquatic animals' performance and feed utilization (Wang et al. 2018). Generally, phytobiotics have appetite-enhancing properties in aquatic organisms, enhancing palatability and regulating appetite control genes in the hypothalamus-pituitary gland (Rao and Kaushik 2021). The positive effects of dietary phytobiotics on growth are associated with their positive effects on the gut microbiota (Suttili et al. 2018). The positive effects of phytobiotics on growth regulation were evidenced by the expression levels of growth factor genes (IGF-1 and IGF-2) at the mRNA level. However, responses can differ greatly depending on the fish species and phytobiotics form (Dawood et al. 2022; Öz et al. 2026).

Certain medicinal plants have been shown in several studies to increase appetite in aquatic animals (Abdel-Aziz et al. 2023 and El-Dakar et al. 2023), act as feeding attractants by chemically improving fish feeding behavior, which increases fish growth, and lower water pollution in culture systems by improving feed utilization (Wang et al. 2018).

Numerous studies have demonstrated that garlic improves the growth performance of aquatic species, such as Nile tilapia (Setijaningsih et al. 2021), rainbow trout *Oncorhynchus mykiss* (Güroy et al. 2024; Öz and Dikel et al. 2022), and Japanese seabass, *Lateolabrax japonicus* (Xu et al. 2020). Meanwhile, it was reported that Japanese seabass was significantly attracted by asa-fetida, which shares a similar taste and smell with garlic, causing improvements in feed utilization and reducing the loss of feed into water, reliably and indirectly showing that garlic powder or oil could contribute to reducing pollution (Zhang et al. 2008; Öz et al. 2024).

Overall, the aquaculture business experiences countless issues due to the rapid development of intensive and ultraintensive systems. These systems are accompanied by negative effects such as rapid deterioration of water quality, increased competition, overcrowding, and faster disease transmission (Fadel et al. 2024; Abdel-Aziz et al. 2024). Consequently, nanotechnology has been used to increase the effectiveness of feed additives from medicinal plants. In another trend, nanomaterials have many advantages, including high absorption and bioavailability, better dispersion and solubility, increased durability against environmental degradation during food preparation, and controlled release kinetics (Pathakoti et al. 2017; Cui et al. 2016). Wherein feeds in the form of NPs, micronutrients can enter cells more quickly and increase the rate of absorption. Also,

Abd El-Naby et al. (2019) found that growth and feed utilization of Nile tilapia can be enhanced by dietary supplementation with chitosan NPs. Abdel-Ghany et al. (2023) reported that adding 50 or 100 mg kg⁻¹ of nanocurcumin to the diet improved performance and mitigated the detrimental effects of heat stress. Additionally, using NPs as delivery vehicles can improve feed's nutritional content and FCR (Bhattacharyya et al. 2015). These benefits increase productivity and quality while lowering waste and financial burden (Chen and Yada 2011).

It was shown that using these NPs can boost the innate immune system in addition to preventing the growth of both aerobic and anaerobic bacteria. However, El Basuini et al. (2022) and Eissa et al. (2023) reported that nanocurcumin is a polyphenolic compound that stimulates the formation of good intestinal flora and may inhibit the growth of microbial and fungal infections, leading to higher feed efficiency.

Garlic's many health benefits are linked to its organosulfur compounds, especially its thiosulfinates, wherein allicin (diallylthiosulfinate) makes up roughly 70% of all the thiosulfinates found in garlic (Block 1992; Drago Serrano et al. 2006; Gambogou et al. 2018). Allicin is a reactive sulfur species causing a redox reaction with thiol groups in glutathione and proteins, a mechanism that is essential for its biological activity (Borlinghaus et al. 2014) and has a potent appetite-stirring and additional digestion-improving impact, which was the main cause of these effects. Furthermore, allicin can improve digestion and the general health of cultured fish by inducing the intestinal flora and inhibiting pathogenic bacteria (Setijaningsih et al. 2021).

Our results showed improvements in the digestive enzymes of fish fed NPsG. In this line, Abd El-Naby et al. (2019) suggested that the addition of chitosan particles boosted the activity of enzymes like lipase and amylase. Similar investigations have shown that raising the activities of digestive enzymes improved the growth performance of Nile tilapia fed with varying concentrations of cinnamaldehyde nanoemulsion (Abd El-Hamid et al. 2021) and NPs cinnamon (Abdel-Tawwab et al. 2018).

Garlic supplementation promotes digestion by boosting bile acid secretion and stimulating enzymes such as lipase, trypsin, alkaline phosphatase, protease, and amylase (Rezaei et al. 2022). Additionally, amylase, lipase, and protease activities were higher in fish-fed or fermented garlic powder than in the control group (El Basuini et al. 2024).

According to previous studies, allicin, which is found in garlic, has positive effects on gut microbiota and may promote better

digestion and energy consumption (Lee et al. 2014). Additionally, garlic can help in the release of bile acids to regulate digestion (Elkayam et al. 2003).

Generally, the state of the gut microbiota is an important factor that can directly affect the host's general health. Fish's intestinal system contains a complex network of microbial communities called the gut microbiota, which is directly responsible for processing food (Liu et al. 2021). Diet has a significant impact on the microbiota's structure (Hoseinifar et al. 2019). Different metabolites are created depending on the microbial activity that is impacted. Digestion may be directly affected by these metabolites. This causes the release of enzymes, particularly intestinal peptides (Butt and Volkoff 2019). It raises the rate and quantity of nutrient absorption, hence influencing fish growth.

The antioxidant capabilities of garlic have been acknowledged for their potential to mitigate the oxidative damage caused by cypermethrin. Garlic oil supplementation has been shown to enhance antioxidant enzyme activities, reduce lipid peroxidation, and maintain the balance of biochemical parameters in fish (Öz et al. 2024). Our results showed an increase in SOD and TAC with a reduction in MDA in groups fed NPsG compared to the control group, especially NPsG600. As is well known, the antioxidant system plays a significant role in immunity and health, and numerous studies have found a good correlation between fish and shellfish immune response and antioxidant activity. SOD is the body's primary frontline defense enzyme against oxidative stress. It actively converts highly reactive, toxic superoxide radicals into harmless oxygen and hydrogen peroxide, safeguarding cell membranes, proteins, and DNA from oxidative damage (Lesser 2006; Zheng et al. 2023; Bao et al. 2022). TAC is a biomarker assay that measures the combined ability of all antioxidants in a biological sample (serum or tissue) to neutralize free radicals. It evaluates the overall, cumulative, and synergistic antioxidant defense system rather than testing individual antioxidants. In addition to measuring TAC, it remains one of the most widely used methods to quantify the possible oxidant-buffering capacity of a sample. Moreover, TAC assays are frequently used to rank antioxidants and to identify structure–activity relationships. Therefore, TAC assays are often employed to search for unknown antioxidants in complex mixtures (Silvestrini et al. 2023; Pillay et al. 2016).

In another trend, MDA does not act as an antioxidant; it is a highly reactive byproduct of polyunsaturated fatty acid (PUFA) peroxidation and is universally utilized as a primary biomarker to measure the severity of oxidative stress and lipid damage in cells (Koruk et al. 2004).

Furthermore, feeding Nile tilapia with nanocurcumin considerably boosted SOD. In the same context, Bao et al. (2022) found that the activity of MDA considerably decreased in the 0.2% NPs curcumin group.

In farmed fish, plant phenolics react as electrophilic molecules that activate the Keap1–Nrf2 (nuclear factor erythroid 2–related factor 2) axis. These substances preserve the stability of Nrf2. Nrf2 activates antioxidant enzymes once it enters the nucleus. This reduces serious oxidative damage in stress in high-intensity fish farming systems (Öz et al. 2026; Cheng et al. 2021).

It is acknowledged that the intestine is the main site for nutrient absorption in fish, and it is where nutrients are transported into and out of the intestinal enterocytes by specific transporters located at the brush border and basolateral membranes (Abdel-Latif et al. 2020). Besides, long villi are typically associated with excellent gut health and high nutrient absorption efficiency, which improves performance (Huerta-Aguirre et al. 2019). In the current study, the addition of NPsG, particularly NPsG600, to the diet of fish markedly increased the length/width of the villi and the area that was absorbed in the mid-intestine in a dose-dependent way. Similarity: Photomicrography of the intestinal tissues in *Oreochromis* sp. given NPs-curcumin shows a notable improvement in the intestinal architecture, including the intestinal villi (El Basuini et al. 2024). To support our data, fish fed garlic-supplemented diets showed increased villi length, branching, and goblet cell number while maintaining normal mucosa and intestinal wall structure. (Abdel-Latif et al. 2024).

Regarding body composition, our results concurred with Bao et al. (2022), who reported no discernible impact of nanocurcumin on the approximate composition of fish muscles and body, including crude protein and crude fat. Also, Xu et al. (2020) discovered that garlic consumption greatly impacted the body crude fat level of Japanese seabass, although the percentages of moisture, crude protein, and ash did not vary among all groups, which is also in agreement with our findings. While Ajiboye et al. (2016) reported that the bioactive compounds in garlic may be responsible for the decrease in the whole-body lipid content of *Tilapia zilli* fish. These compounds increase the excretion of acidic and neutral steroids, which lower the cholesterol level in the fish's body composition. Additionally, they mentioned that the significantly increased protein content of fish carcasses might be caused by the experimental fish's higher amount of appetizing amino acids, which came about because of intestinal proteases being activated by the garlic's allicin compound.

Regarding the dose–response pattern, many parameters showed a bell-shaped response (improving from 0 to 600 mg/kg, then declining at 1200 mg/kg), as shown in Figure 2A. This may be because the effectiveness of doses is affected by several factors, including the physicochemical characteristics of the NPs (size and surface area), surface charge, and coating, which play a role in particle aggregation and stability, and exposure duration. Excessive concentrations can be detrimental, while low to moderate amounts promote growth. Also, optimal dosages depend on exposure duration; low doses may not have an impact during brief rearing times. Growth stage and species also influence NPs' sensitivity and absorption capacities differ depending on both growth stage and species (Khosravi-Katuli et al. 2017; Paolino et al. 2021).

Due to their small size and high surface area-to-volume ratio, NPs can interact uniquely with biological systems (Abbasi et al. 2024); the risk of cell penetration, oxidative stress, and bioaccumulation increases if the dosage is too high. The NPs' toxicity is also influenced by the above-mentioned factors (Paolino et al. 2021). The disruption of the natural equilibrium between oxidative stress and antioxidant defense indices may be one of these detrimental impacts, which can then result in several diseases (Shine et al. 2022). Confirming this, El-Saadony et al. (2021) reported that overuse of NPs at high concentrations can lead

to oxidative stress, cellular damage, and bioaccumulation in aquatic organisms. These observations were in agreement with our data in Figure 3A–C. Additionally, SeNPs in high concentrations decreased sperm quality, increased oxidative stress and DNA damage in sperm, and disrupted testis development (Ibrahim et al. 2021). This may reflect the growth reduction in fish fed 1200 mg/kg of NPsG. Similarly, the addition of nanocurcumin, at the highest level (200 mg kg⁻¹ diet), decreased growth performance and feed utilization of tilapia compared to control (Abdel-Ghany et al. 2003). In fact, phytobiotics such as garlic or curcumin, which are polyphenols that can bioaccumulate in body tissues at high levels, may cause growth regression (Omnes et al. 2017).

5 | Conclusion

In summary, when utilized as a feed additive, nanotechnology-based medicinal plants or herbal substances can be more beneficial for fish nutrition by stimulating digestive enzymes, improving nutrient absorption and bioavailability, and improving intestinal histomorphometry. The results of this study, which looked at various dosages of NPsG as supplemental feed, showed that 600 mg/kg of NPsG had the best physiological state and, consequently, the highest performance. While high levels of NPsG (1200 mg/kg) had adverse effects. Should highlight practical implications (commercial feed, cost-effectiveness) As a recommendation for future studies, improving the efficiency of feed additives by converting them to NP forms requires further research, both on the most effective preparation method and on the appropriate dosages to be administered to fish. In the present study, the optimum dose range of NPsG was 300–600 mg/kg, which requires validation on a larger scale. Also, limitations to be considered in future studies include health/immunological challenges and allicin content of NPsG compared to garlic powder, as well as with the extension of the feeding period.

Author Contributions

Sami Alaa Sami; Ashraf Y. El-Dakar; Abdelwahab A. Abdelwarith; Elsayed M. Younis; Mohammed. F. El Basuini; Shymaa M. Shalaby; Mohamed M. Rashad, Simon J. Davies, Mohamed F. Abdel-Aziz: conceptualization, data curation, formal analysis, investigation, methodology, resources, validation, visualization. **Mohammed F. Abdel-Aziz:** writing – original draft, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data available on the request from the authors.

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