Effective role of dietary curcumin nanoparticles and Spirulina platensis supplementation on growth, digestive enzymes, hematological, serum biochemical parameters, antioxidant status, immune responses, and histological examination in Nile tilapia fingerlings

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Abstract

The positive effects of feeding nano-curcumin and Spirulina platensis on *O. niloticus*'s growth performance, hepatoprotective ability, antioxidant response, immunity, digestibility, and organ histopathology were predicted by this study. Nile tilapia fingerlings (n = 180 fish; 10 ± 0.5 g) were divided into three groups (three replicates/group) and fed diets containing zero percent (control), 30 mg kg-1 curcumin nanoparticles (Cu-NPs group), and 5 g kg-1 *Spirulina platensis* (SP group). Growth performance, haematological, antioxidant, biochemical, and immunological markers plus histological changes were evaluated after the feeding study lasted 56 days. Our results revealed that Cu-NPs and SP have favorable effects on carcass composition, growth performance, hematological, and biochemical parameters. Moreover, Cu-NPs and SP supplementation significantly elevated serum Ig M level ($p < 0.01$, $p < 0.05$), lysozyme ($p < 0.001$), amylase ($p < 0.05$, $p < 0.01$), lipase ($p < 0.05$) and protease ($p < 0.01$) activities unlike the control group. Additionally, significant elevation of SOD ($p < 0.001$) and GPx ($p < 0.01$, $p < 0.05$) activities with diminished MDA ($p < 0.001$, $p < 0.01$) production was noticed in SP and Cu-NPs groups compared to control one. Overall, diets supplemented with Cu-NPs and SP effectively improved the growth performance, hepatic function, immune response, and antioxidant impacts in Nile tilapia fingerlings.

1. Introduction

Nile tilapia, *O. niloticus* is a well-known aquaculture species in wide nations, including Egypt, where it makes up about 75.54% of all aquaculture production. After China and Indonesia, Egypt produces the third-largest amount of tilapia in the world (Abdel-Tawwab et al., 2022; FAO, 2020; Fitzsimmons, 2016). The culture of Nile tilapia has many difficulties including bacterial infection and environmental stressors are the biggest dangers to successful production (Aly et al., 2016).

Recently, natural phytogenic substances are frequently utilized in animal diets as antimicrobials and growth promoters, especially in aquaculture (Sruthi et al., 2018) to minimize the use of antibiotics that cause bacterial strains resistant (Nm et al., 2018) and their accumulation in edible tissues (Grenni et al., 2018). In order to increase development and improve disease resistance, researchers are currently emphasizing the usage of non-antibiotic and natural growth promoters for the enhancement of the gastrointestinal tract by the addition of certain feed additives that have antibacterial, antioxidant, anti-inflammatory, and appetite-stimulating, immune-modulating and gastroprotective impacts on animal health (Moniruzzaman and Min, 2020).

Curcumin is hydrophobic and polyphenolic compound obtained from turmeric (*Curcuma longa* L.) and known as diferuloylmethane [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], that its dried powder consisted of 60–70% carbohydrate, 6–8% protein, 6–10% fat, 3–6% fiber, 3–6% and terpenes and terpenoids (Prasad et al., 2014; Singh et al., 2010). Because of the extensive range of pharmacological properties it possesses, such as its lipid-lowering, anti-inflammatory, antioxidant, anticancer, and antiviral effects (Alagawany et al., 2021; Ji et al., 2021; Nm et al., 2018), curcumin has consistently garnered a lot

of attention in aquaculture. Recent studies have shown that adding curcumin to Nile tilapia diet can improve growth performance and disease resistance (Abdel-Tawwab et al., 2022; Ajani et al., 2020). As well, dietary curcumin supplementation could enhance *Oncorhynchus mykiss* development, immune response, and antioxidant potential (Yonar et al., 2019). Curcumin also could lessen the apoptosis and liver damage caused by chlorpyrifos in largemouth bass (Zhao et al., 2021). Despite curcumin's advantages, it has certain limitations, including it is poorly absorbed in the body due to its low water solubility (hydrophobic) and unstable molecular structure, additionally, its bioavailability or utilization varies according to sex and species (Kharat and McClements, 2019). That's why, it has been extensively documented that lipophilic curcumin can be converted into nanoparticles to increase its bioavailability and solubility (Ghalandarlaki et al., 2014). Nanocurcumin had a better solubility and absorption rate than the regular version of the compound (Hani and Shivakumar, 2014; Kurita and Makino, 2013). A previous report clarified that dietary intake of 0.2% nano-curcumin significantly enhanced the mucosal immune system, antioxidant capacity, and glucose metabolism of largemouth bass (Bao et al., 2022). Moreover, Nile tilapia fingerlings' antioxidant potential, humoral immunity, as well as hepatic and intestinal histology were all improved by dietary supplementation of curcumin nanoparticles (Abdel-Tawwab et al., 2022). However, research on nano-curcumin in fish is still needed and requires additional investigation, especially in Nile tilapia.

Microalgae are known as filamentous creatures in freshwater and marine habitats. They have been used as food and animal feed for many years (Araújo et al., 2021; Vigani et al., 2015). Blue-green microalga spirulina (*Arthrospira platensis*) is a filamentous cyanobacterium that has a high nutritional profile with 60–70% proteins and vitamins (Ahsan et al., 2008). As well, it has been determined to be a viable source of protein for farmed fish (Krishnaveni et al., 2013). Phycocyanin and allophycocyanin are two pigments found in the spirulina, which also contains essential amino acids, polyunsaturated fatty acids (PUFA), protein, B vitamins (primarily riboflavin), necessary minerals (primarily iron), minerals, carotenoids, chlorophyll, and other dietary components. (Güroy et al., 2012; Palmegiano et al., 2008). According to numerous research on Nile tilapia supplementation of spirulina significantly improved growth rates, immune response, and disease resistance (Abdel-Tawwab and Ahmad, 2009; Amer, 2016; Awad et al., 2022; Mahmoud et al., 2018). *S. platensis* is also recorded to have beneficial impacts on disease resistance and immunity of African catfish *Clarias gariepinus* (Promya and Chitmanat, 2011), due to its content of pigments that have antioxidative valuables and can scavenge free radicals. Considering the advantages of the curcumin and spirulina, the current study aimed to assess the beneficial impacts of nano-curcumin and spirulina supplementation on growth performance, hepatoprotective capacity, antioxidant, immunity, digestibility, and organ histopathology of Nile tilapia fingerlings.

2. Materials And Methods

2.1. Curcumin nanoparticles (Cu-NPs) preparation

An approach was followed to manufacture C-NPs utilizing a syringe pump containing antisolvent, with a few minor adjustments (Kakran et al., 2012). The organic solvent employed was dichloromethane

(Carvalho et al., 2015). The initial curcumin solution was made in dichloromethane (5 mg/mL), placed in a 20-mL syringe, and then injected (10 mL/min) at a 1:12 ratio into deionized water (the antisolvent) while being stirred magnetically (1000 g) for two hours. Filtered and vacuum-dried we applied on the produced NPs. A Zeta sizer was used to determine the Cu-NP dimension (Malvern Instruments, Zeta sizer nano series Nano-s, UK). The particles' average diameter was 82.7 ± 11.1 nm (Figure. 1).

2.2. Dietary preparation

Three diets that are isonitrogenous (32 percent) and isocaloric (3000 Kcal DE/kg) were developed to meet the dietary needs of Nile tilapia (*O. niloticus*) according to nutrient requirements of fish (NRC, 2011). In this investigation, a control baseline diet, a basal diet with Cu-NPs (30 mg kg^{-1}) (Abdel-Tawwab et al., 2022), and a basal diet with *Spirulina Platensis* (SP) (5 g kg^{-1}) (Teimouri et al., 2019) were all used as dietary treatments. The components of each dietary regimen were listed (Table. 1). All diets were made as water-stable sinking pellets and kept in the refrigerator in plastic bags while being consumed.

2.3. Fish rearing

Nile tilapia fingerlings (n = 180 fish; 10 ± 0.5 g) were housed for two weeks in order to adapt and adjust to the laboratory setting. Fish were fed the control diet (30 percent CP) three times daily, up until apparent satiety. After acclimatization, fish were distributed at random into 15 100-L tanks with a density of 20 fish per tank to represent 3 treatments in triplicates. Using air pumps and air stones, compressed air was delivered to fish tanks. Fish were fed on the test diets three times per day at 9:00 h, at intervals of four hours, for a total of 56 days, or until they were satisfied. Every day, waste was evacuated from each tank and half of it was replaced with fresh water.

Throughout the trial period, fluorescent light tubes were used to maintain a 12 h: 12 h cycle of light and dark. Water temperature, dissolved oxygen, pH level, and un-ionized ammonia were measured twice daily on the sites using an automatic probe (Hanna HI-9147). The measurements' ranges were, respectively, 27.0-29.2°C, 6.2–6.9 mg/L, 7.95–8.37 mg/L, and 0.011–0.21 mg/L. The optimal ranges for each of these variables for fish culture are present (Boyd and Tucker, 1998). After two weeks, fish were randomly divided into four groups and placed in glass aquarium tanks (40×60×30 cm) (20 Fish/tank) with dechlorinated tap water and aeration. Each group received a set of three tanks, totaling 60 fish per group.

The groups received the following treatments for 56 days; the control group received a control basal diet. Curcumin nanoparticles were added to the diet of the Cu-NPs group of fish at a level of 30 mg kg^{-1}. Fish in the SP Group were given a meal comprising 5 g kg^{-1} of *Spirulina platensis*.

2.4. Sample collection and preparation of tissue homogenates

Fish were sampled 24 hours after the fish had stopped eating as part of the feeding trial. Each fish's body size and length were recorded, and the growth parameters and a few biological indices were computed in accordance. Following anesthesia with clove oil (60 mg L^{-1}) on five fish from each group, blood samples

were taken from the caudal blood vessels of each individual fish (Simões et al., 2011). Disposable syringes with dipotassium EDTA solution were used to draw one blood sample for blood counting. The serum was separated from the other blood sample using centrifugation at 3000 g for 15 min at 4°C, and it was then stored at 80°C pending the investigation of some biochemical, immunological, and antioxidant indices. This blood sample was drawn using non-heparinized disposable syringes. To obtain the previously mentioned samples (gills, stomach, intestine, liver, and kidney) the fish belly was dissected (Pirarat et al., 2011).

2.5. Growth performance, feed utilization indices, and survival percent

Fish were removed from each tank at the end of the experiment, numbered, and weighed. The formulas described below were used to calculate the feed utilization indices and parameters for fish growth (Abdelghany et al., 2020; Yıldız et al., 2006):

Weight gain % = 100 ($W_2 - W_1$) / W_1;

Specific growth rate (SGR; %g/day) = 100 [Ln W_2 (g) – Ln W_1 (g)] / T; where W_2 is final weight (g), W_1 is initial weight (g), and T is the trial period (day)

Feed conversion ratio (FCR) = feed intake / WG;

Fish survival (%) = 100 (fish number at end of experiment / fish number at start).

2.6. Carcass composition analysis

For the initial and final proximate carcass studies, samples of eight fish were collected at the time of harvest and stocking, respectively. The proximate analysis was completed using the accepted procedures of (Helrich, 1990). The nutrients dry matter (DM), crude protein (CP), ether extract (EE), and ash were examined. Kjeldahl nitrogen was used to measure crude protein, and weight loss following petroleum ether extraction of the sample (40–60°C) was used to quantify crude fat. Dry samples were burned for four hours at 550°C in a muffle furnace to estimate the amount of ash.

2.7. Hematological studies

Erythrocytes and leukocytes were manually counted after being diluted in Natt- Herrick's solution (Hrubec et al., 1996). Additionally, hemoglobin, packed cell volume, and red blood cell indices were assessed using these modulated approaches (Drabkin, 1946; Jain, 1986). Wright's Giemsa stain was also applied to blood films in order to determine the differential leukocytic count (Hrubec et al., 1996).

2.8. Serum biochemical assays

Fish blood glucose level was assessed following the a previous modified method (Wedemeyer and Yasutake, 1977). Levels of cholesterol (Biotrend Co., Maryland, USA, Catalog No.; EK12283), triglycerides (Clinisciences Co., Nanterre, France) Catalog No.; AE63342FI-4800), total protein (MyBioSource Co.,

California, USA, Catalog No.; MBS9917835), and albumin (MyBioSource Co., California, USA, Catalog No.; MBS019237), as well as, activities of aspartate aminotransferase (AST, Biotrend Co., Maryland, USA, Catalog No.; EK12276), alanine aminotransferase (ALT, MyBioSource Co., California, USA, Catalog No.; MBS038444) and alkaline phosphatase (ALP, MyBioSource Co., California, USA, Catalog No.; MBS033204) were determined according to the principle of their particular pamphlets using a spectrophotometer (Lambda EZ201; Perkin Elmer). Moreover, the creatinine and uric acid concentrations were measured as previously described (Bartles et al., 1972; Prætorius and Poulsen, 1953; Wedemeyer and Yasutake, 1977), respectively.

2.9. Immune parameters

Based on the lysis of *Micrococcus lysodeikticus*, serum lysozyme activity was evaluated (Ghareghanipoora et al., 2014), with only minor alterations (Sigma Co., USA). The optical density was then assessed every minute for five minutes at 540 nm. To calculate the serum lysozyme content, a calibration curve was created using various dilutions of lyophilized chicken egg-white lysozyme (Sigma Co., USA). According to the manufacturer's instructions, the serum levels of immunoglobulin M (IgM, Catalog No.; CSB-E12045Fh) were also measured using their kits bought from Cusabio Co. (Texas, USA).

2.10. Intestinal digestive enzymes activity

The amylase, lipase, and protease enzyme activities of the intestinal homogenate supernatant were determined using the techniques of other investigations (Bernfeld, 1955; Hidalgo et al., 1999; Ono and Iijima, 1998), respectively.

2.11. Oxidative stress/antioxidant status

According to previously described techniques, serum from each group was utilized to roughly calculate the superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) levels (Aebi, 1984; Benzie and Strain, 1996; Ellman, 1959; Nishikimi et al., 1972; Ohkawa et al., 1979).

2.12. Histopathological analyses

In 10% neutral buffered formalin, specimens from the gills, stomach, intestine, liver, and kidney were preserved. The tissues were then processed using ethanol and xylene in increasing concentrations, embedded in paraffin, and sectioned using a rotary microtome into 4 µm thick sections. Hematoxylin and eosin were used to stain tissue sections, and a light microscope with a digital camera was used to examine and photograph the tissue (Olympus, Tokyo, Japan).

2.13. Statistical Analysis

The statistical analyses of growth performance, body composition, haematological parameters, and biochemical evaluation tests were performed using the SPSS® programme, version 26.0 (IBM Corporation, SPSS Statistics, USA). The results of the trial were presented as means ± SEM. All data were initially checked for variance, normality, and homogeneity using Levene's test. Then, to compare the mean values across the experimental groups, a one-way ANOVA was used with post-hoc Duncan's multiple range tests. P 0.05 was regarded as statistically significant. One-way ANOVA with Post-hoc Tukey's multiple range testing were used to examine the data on digestive, immune, and antioxidant enzymes (*p < 0.05, **p < 0.01; ***p < 0.001), and GraphPad Prism version 8.0 was used to depict the results graphically (GraphPad Software, Inc., USA).

3. Results

3.1. Effect of Cu-NPs and SP supplementation on growth performance, feed utilization indices, and survival percent

Table 2 summarises the growth performance, feed consumption, and survival of fish fed diets enriched with Cu-NPs and SP. Compared to fish fed control diets, fish fed Cu-NPs and SP supplemented diets significantly improved (p < 0.05) the FBW, WG, SGR, feed intake, and fish biomass. Fish from the Cu-NPs and SP groups had significantly lower FCR (p < 0.05) than the control fish. Additionally, there were no notable changes in survival percentages between any of the experimental groups (p > 0.05).

Table 1
Composition and proximate analysis of basal and supplemented diets (% dry matter) of *O. niloticus* with curcumin nanoparticles (Cu-NPs) and *Spirulina platensis* for 56 days

Ingredients	Cont.	Cu-NPs (30 mg kg^{-1})	SP (5 g kg^{-1})
Fish meal (CP 72%)	110.00	110.00	110.00
Soybean meal (CP 48%)	360.00	360.00	360.00
Rice bran	200.00	200.00	200.00
Wheat bran	200.00	200.00	200.00
Yellow corn	60.00	59.970	55.0
Nano Se	----	0.030	----
Spirulina	----	----	5.0
Fish Oil	15.00	15.00	15.00
Soybean Oil	15.00	15.00	15.00
Molasses	20.00	20.00	20.00
Dicalcium phosphate	10.00	10.00	10.00
Vit [1] & Min [2] premix	10	10	10
Total	1000	1000	1000
Proximate analysis (% dry matter basis)			
Dry matter	91.5	91.48	91.42
Crude Protein	30.29	30.28	30.32
Crude Fat	8.17	8.16	8.17
Crude Fiber	6.62	6.61	6.61
Carbohydrate (NFE)[2]	47.70	47.71	47.70
Ash	7.23	7.21	7.22
Gross energy kcal/100g3	3.742	3.734	3.722

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

[2] Mineral premix (g/kg of premix): CaHPO4.2H2O,727.2; MgCO4.7H2O, 127.5; KCl 50.0; NaCl, 60.0; FeC6H5O7.3H2O, 25.0; ZnCO3, 5.5; MnCl2.4H2O, 2.5; Cu(OAc)2.H2O, 0.785; CoCl3.6H2O, 0.477; CaIO3.6H2O, 0.295; CrCl3.6H2O, 0.128; AlCl3.6H2O, 0.54; Na2SeO3, 0.03

Table 2
Growth performance and feed utilization of Nile tilapia (*O. niloticus*) fed diets with supplemented with curcumin nanoparticles (Cu-NPs) and *Spirulina platensis* for 56 days (n = 3).

Parameters	Cont	Cu-NPs (30 mg kg^{-1})	SP (5 g kg^{-1})
Initial weight (g)	10.00 ± 0.06^{a}	9.97 ± 0.03^{a}	10.00 ± 0.06^{a}
Final weight (g)	24.77 ± 0.25^{b}	35.85 ± 1.43^{a}	37.43 ± 1.63^{a}
Weight Gain (g)	14.77 ± 0.28^{b}	25.88 ± 1.46^{a}	27.43 ± 1.69^{a}
Weight Gain %	147.67 ± 3.38^{b}	259.67 ± 15.32^{a}	274.57 ± 18.48^{a}
SGR (%g/day)	1.62 ± 0.03^{b}	2.28 ± 0.08^{a}	2.45 ± 0.05^{a}
Feed intake (g feed/fish)	30.59 ± 0.13^{b}	33.63 ± 0.51^{a}	34.13 ± 0.37^{a}
FCR	2.07 ± 0.03^{a}	1.31 ± 0.05^{b}	1.25 ± 0.06^{b}
Fish biomass (g fish)	495.33 ± 4.91^{b}	705.87 ± 38.55^{a}	748.67 ± 32.62^{a}
Survival rate (SR %)	100.00 ± 0.00^{a}	98.33 ± 1.67^{a}	100.00 ± 0.00^{a}

***Values are expressed as means ± SE (n = 3). Data in the same row assigned with the different superscripts are significantly different ($p < 0.05$) using ANOVA Post Hoc (Duncan test). FCR, feed efficiency ratio; SGR, specific growth rate; SR, survival rate.**

3.2. Effect of Cu-NPs and SP supplementation on carcass composition analysis

Table 3 shows the carcass-proximate composition of *O. niloticus* fed spirulina (SP) and curcumin nanoparticles (Cu-NPs). Fish fed a diet containing Cu-NPs and SP had significantly higher levels of dry matter (DM), protein, and ash ($p < 0.05$) than fish fed a control diet. In terms of ether extract (%), there was no discernible difference between the experimental groups.

Table 3
Carcass proximate composition of O. niloticus fed with curcumin nanoparticles (Cu-NPs) and *Spirulina platensis* for 56 days (n = 3).

Compositions (g kg^{-1})	Cont	Cu-NPs (30 mg kg^{-1})	SP (5 g kg^{-1})
DM (%)	25.25 ± 0.05^{c}	27.33 ± 0.10^{a}	27.01 ± 0.05^{b}
Protein (%)	57.13 ± 0.55^{c}	59.63 ± 0.06^{b}	60.94 ± 0.08^{a}
Ether Extract (%)	25.49 ± 0.60^{a}	24.37 ± 0.07^{a}	24.61 ± 0.05^{a}
Ash (%)	14.44 ± 0.05^{c}	15.21 ± 0.04^{b}	15.40 ± 0.02^{a}
***Values are expressed as means ± SE (n = 3). Data in the same row assigned with the different superscripts are significantly different (p < 0.05) using ANOVA Post Hoc (Duncan test).**			

3.3. Effect of Cu-NPs and SP supplementation on hematological analysis

Fish treated with Cu-NPs and SP showed a substantial increase ($p < 0.05$) in RBC count, Hb concentrations, and Ht% when compared to control fish. MCH, however, did not reveal any group differences that were significant ($p > 0.05$). Compared to the SP and control groups, total leukocyte (WBC) counts were considerably higher ($p < 0.05$) in fish treated with Cu-NPs. Additionally, Cu-NPs and SP groups' neutrophils and monocytes were significantly enhanced ($p < 0.05$), while the control group's lymphocytes were dramatically decreased ($p < 0.05$) (Table 4).

Table 4
Hematological parameters of Nile tilapia (*O. niloticus*) fed diets supplemented with curcumin nanoparticles (Cu-NPs) and *Spirulina platensis* for 56 days.

	Cont	Cu-NPs & Spirulina (mg kg^{-1} diet)	
		Cu-NPs	Spirulina
RBCs (1x10^6 uL)	3.12 ± 0.05^b	3.73 ± 0.02^a	3.64 ± 0.08^a
Hb (mg/dL)	7.37 ± 0.42^b	9.37 ± 0.19^a	8.83 ± 0.26^a
MCH	23.60 ± 1.42^a	25.14 ± 0.61^a	24.52 ± 1.07^a
Ht (%)	39.00 ± 2.80^b	50.93 ± 0.23^a	48.50 ± 2.72^a
WBCs (x10^3 uL)	18.13 ± 0.94^b	22.07 ± 1.12^a	19.40 ± 0.53^{ab}
Lymphocytes (%)	65.37 ± 0.74^a	51.23 ± 1.96^b	54.53 ± 1.10^b
Neutrophils (%)	22.50 ± 0.61^b	30.47 ± 1.60^a	28.20 ± 1.10^a
Monocytes (%)	11.40 ± 0.10^b	19.20 ± 0.51^a	18.30 ± 1.10^a

***Values are expressed as means ± SE (n = 3). Data in the same row assigned with the different superscripts are significantly different (p < 0.05) using ANOVA Post Hoc (Duncan test).**

3.4. Effect of Cu-NPs and SP supplementation on serum biochemical parameters

The total impacts of Cu-NPs and SP supplementation serum biochemical markers of *O. niloticus* were demonstrated in Table 5. Cu-NPs and SP supplementation of significantly reduced ($p < 0.05$) the serum glucose and cholesterol levels of *O. niloticus* relative to that of the control fish. As well, triglycerides showed significantly higher value ($p < 0.05$) in SP group unlike Cu-NPs and control groups. Moreover, significantly higher ($p < 0.05$) levels of total protein, albumin and globulin in both SP and Cu-NPs groups versus the control group. AST and ALP activities were also lessen in SP and Cu-NPs groups, unlike the control group. No statistical changes ($p > 0.05$) were detected ALT, uric acid, and creatinine in the serum of *O. niloticus* of all experimental groups.

Table 5
Changes in haemato-biochemical parameters of Nile tilapia (*O. niloticus*) fed diets supplemented with of curcumin nanoparticles (Cu-NPs) and *Spirulina platensis* for 56 days.

	Cont	Cu-NPs & Spirulina (mg kg^{-1} diet)	
		Cu-NPs	SP
Glucose (mg/dL)	113.80 ± 5.08^{a}	89.37 ± 0.90^{b}	93.47 ± 2.43^{b}
Total cholesterol (mg/dL)	192.67 ± 5.24^{a}	180.17 ± 2.37^{b}	173.43 ± 0.29^{b}
Triglycerides (mg/dL)	152.77 ± 2.39^{b}	147.53 ± 3.67^{b}	170.43 ± 2.43^{a}
Total protein (g/dL)	4.09 ± 0.04^{c}	5.22 ± 0.04^{b}	5.77 ± 0.03^{a}
Albumin (g/dL)	2.36 ± 0.01^{c}	2.49 ± 0.02^{b}	2.77 ± 0.02^{a}
Globulin (g/dL)	1.73 ± 0.03^{c}	2.73 ± 0.02^{b}	3.00 ± 0.01^{a}
Alb\Glob	1.36 ± 0.02^{a}	0.91 ± 0.00^{b}	0.92 ± 0.00^{b}
AST (IU/L)	12.40 ± 0.10^{a}	12.13 ± 0.09^{ab}	11.87 ± 0.09^{b}
ALT (IU/L)	41.87 ± 0.91^{a}	42.20 ± 0.90^{a}	39.43 ± 0.84^{a}
ALP (IU/L)	31.20 ± 0.25^{a}	30.33 ± 0.24^{b}	28.57 ± 0.20^{c}
Creatinine (mg/dL)	1.75 ± 0.10^{a}	1.81 ± 0.03^{a}	1.81 ± 0.02^{a}
Uric acid (mg/dL)	0.53 ± 0.01^{a}	0.53 ± 0.01^{a}	0.52 ± 0.02^{a}

***Values are expressed as means ± SE (n = 3). Data in the same row assigned with the different superscripts are significantly different (p < 0.05) using ANOVA Post Hoc (Duncan test).**

3.5. Effect of Cu-NPs and SP supplementation on immune parameters

As clarified in Fig. 2, Cu-NPs and SP supplementation enhanced fish immunity which was identified by significant elevation of serum lysozyme activity ($p < 0.001$) and Ig M ($p < 0.01$, $p < 0.05$) dissimilar to the control group.

3.6. Effect of Cu-NPs and SP supplementation on serum digestive enzymes activities

Fish fed Cu-NPs and SP-containing diet showed significantly elevated serum amylase ($p < 0.05$, $p < 0.01$), lipase ($p < 0.05$), and protease ($p < 0.01$) activities in contrast to the control regime (Figure. 3), suggesting

the effective role of Cu-NPs and SP supplement in improving the feed efficiency and growth of fish.

3.7. Effect of Cu-NPs and SP supplementation on oxidative/antioxidant status

The administration of diets containing Cu-NPs and SP enhanced antioxidant status, as represented in Figure. (4). Significant elevation of SOD ($p < 0.001$) and GPx ($p < 0.01$, $p < 0.05$) activities with diminished MDA ($p < 0.001$, $p < 0.01$) production in SP and Cu-NPs groups compared to control one (Fig. 4A, C, D). Meanwhile, serum CAT ($p < 0.05$) improved significantly in fish fed Cu-NPs incorporated diets relative to the SP and control groups (Figure. 4B).

3.8. Effect of Cu-NPs and SP supplementation on gills, stomach, intestinal, hepatic, and renal histological findings

Microscopy of the gills in the control group revealed severe diffuse hyperplasia of lamellar epithelium with the fusion of the secondary gill lamellae (Figure. 5a). In the Cu-NPs group, the hyperplasia of the lamellar epithelium (Figure. 5b) was less severe compared to control. In the SP group, the gills had moderate hyperplasia of the gill epithelium (Figure. 5c).

In the control group, microscopy of the stomach revealed focal leukocytes infiltration and fibrosis in the gastric submucosa (Figure. 5d). Meanwhile, less severe leukocytes infiltration and fibrosis (Figure. 5e) were detected in Cu-NPs group, and mild histopathological alteration in the stomach of SP- supplemented group (Figure. 5f).

The intestine revealed focal leukocyte infiltration in the submucosa and focal epithelial hyperplasia in the control group (Figure. 5g). These lesions however were not observed in the Cu-NPs and SP groups (Figure. 5h, i). Liver microscopy showed mild periportal leukocyte infiltration and moderate hepatocyte vacuolation (Figure. 5j). In Cu-NPs and SP groups, the hepatocyte vacuolation was more severe compared to the control group (Figure. 5k, l). Microscopy of the kidney showed mild histopathological alteration in all experimental groups (Figure. 5m, n, o).

4. Discussion

Using natural nutraceuticals is a key strategy for aquafeed sustainability (Paray et al., 2021; Zhu, 2020). Diverse of medicinal herbal plants and microalgae have been validated as growth-promoting and immunostimulant agents in aquaculture (Adel et al., 2021; Mahmoud et al., 2018; Mohammadiazarm et al., 2021; Zahran et al., 2020; Zahran et al., 2021; Zhu, 2020). In this study, fish fed Cu-NPs and SP containing diets for 56 days exhibited pronounced improvements in the growth rate, SGR, and FCR of Nile tilapia fed Cu-NPs and SP. Dietry including of Cu-NPs has resulted in enhanced growth performance in Nile tilapia (*O. niloticus*) (Abdel-Tawwab et al., 2022), white leg shrimp (*Litopenaeus vannamei*) (Bhoopathy et al., 2021). Besides, dietary inclusion of curcumin improved the growth parameters in gilthead seabream in a dose-dependent manner for 150 days (Ashry et al., 2021). Enhanced growth

performance was also reported in the juveniles of rainbow trout (Akdemir et al., 2017), common and grass carp fed curcumin diets (Giri et al., 2021; Ming et al., 2020). The curcumin growth-promoting effect could be related to its enhanced digestion activity via increasing the activities of lipase, trypsin, and amylase enzymes in intestine and hepatopancreas to facilitate absorption of nutrients and consequently amended nutrient utilization and feed efficiency (Abd El-Hakim et al., 2020; Jiang et al., 2016). Curcumin has an attractive flavor, concurrently it could augment the palatability of feed and therefore increased the feed intake (Alagawany et al., 2021).

It is well described that the dietary supplementation of Spirulina *Arthrospira platensis* has enhanced the growth performance of Nile tilapia (Awad et al., 2022), Caspian brown trout (Roohani et al., 2019), as well as Oscar fish, *Astronotus ocellatus* (Mohammadiazarm et al., 2021). These outcomes are attributed to the high contents of bioactive compounds in spirulina, involving polyunsaturated fatty acids, mainly *γ*-linolenic acid, polysaccharides, and also pigments such as total carotenoids; Zeaxanthin, *β*-carotene, chlorophyll, and phycocyanin (Awad et al., 2022; Roohani et al., 2019; Rosas et al., 2019). Furthermore, improved digestive enzyme activities in the intestine of aquatic species were assayed by dietary spirulina (Rosas et al., 2019; Teimouri et al., 2013). In this regard, the incorporation of Cu-NPs and SP in diets of Nile tilapia induced the serum activities of amylase, lipase, and protease enzymes. In similar studies, curcumin supplementation increased the activities of digestive enzymes as represented in fingerlings of *Oreochromis mossambicus* (Midhun et al., 2016), crucian carp (Jiang et al., 2016), and gilthead seabream larvae (Xavier et al., 2021). Recently, increasing the efficacy of digestive enzymes activities has improved the growth performance of Nile tilapia fed with graded levels of Cu-NPs for 60 days (Abdel-Tawwab et al., 2022). Moreover, dietary SP improved the digestive enzyme activities in the intestine of rainbow trout (*Oncorhynchus mykiss*) (Teimouri et al., 2013; Teimouri et al., 2016), and Oscar fish, *Astronotus ocellatus* (Mohammadiazarm et al., 2021), resulting in enhanced feed intake and decreased nutrient retention.

In the present study, fish body protein and ash contents were increased by dietary supplementation of Cu-NPs and SP after 56 days of feeding trial with no marked change in the lipid content. Recent studies demonstrated that long-term feeding with curcumin markedly enhanced the protein and ash contents of Nile tilapia muscles (Abd El-Hakim et al., 2020; Amer et al., 2022). Similar results were reported in Oscar fish, *Astronotus ocellatus* fed SP powder (Mohammadiazarm et al., 2021). Dietary SP increased the ash content of whole body and dorsal muscles along with significant decreased in lipid content in the juvenile gibel carp (Cao et al., 2018b). Data of growth performance with Cu-NPs and SP diets were confirmed by those findings.

Hematological features are reliable tools to assess the impact of herbal products on the health and nutritional status of fish (Vazirzadeh et al., 2017; Yonar et al., 2019). Our current study displayed that fish fed on Cu-NPs and SP diets improved RBCs and WBCs counts, including neutrophils and monocytes, as well as Hb concentration and Ht level compared with the control fish with no significant change in MCH level. These findings are consistent with previous researches, which assayed the positive effects of dietary curcumin and its nanoparticles analogues on the hematological parameters in a variety of fish

species (Abdel-Tawwab et al., 2022; Ashry et al., 2021; Mohamed et al., 2020; Yonar et al., 2019). The enhanced hematological indices emphasized the salutary role of curcumin nanoparticles and spirulina on the health of Nile tilapia with no anemic symptoms with promoted mechanism of erythropoiesis and hemosynthesis, indicating the positive link of curcumin to the metabolic functions and availability of nutrients in the blood of Nile tilapia (Ashrafizadeh et al., 2020; Ashry et al., 2021; Fazio, 2019). Besides, increasing the percentages of neutrophils and monocytes in curcumin and Cu-NPs diets are associated with ameliorated properties of both innate and adaptive immune system of fish (Abdel-Tawwab et al., 2022; Moniruzzaman and Min, 2020; Pereira et al., 2020). Curcumin can probably activate the neutrophils and macrophages, to release reactive oxygen species (ROS) subsequent with increased the phagocytic activity, which is involved in immunity (Ashry et al., 2021; Jagetia and Aggarwal, 2007). In this regard, *spirulina platensis* had also ameliorated Hb, HCT, MCH and MCHC in Oscar fish (Mohammadiazarm et al., 2021), and this improvement is attributed to phycocyanin of *spirulina platensis* is known for its beneficial effects on the stem cells in the bone marrow, which promoted the cellular immune system and red blood cells of fish (Meng-umphan, 2008).

Lysozyme is a substantial hydrolytic enzyme of the innate immunity of fish, causing lysis of bacterial pathogens with triggering of the complement system and phagocytes (Saurabh and Sahoo, 2008). Serum immunoglobulins play a vital role in phagocytosis and killing of pathogenic microrganisms inside the fish body (Magnadottir, 2010). Our existing data indicated remarkable increase in lysozyme, and total Ig levels in Nile tilapia fed with C-NPs and SP. Curcumin administration showed marked augmentation of IgM values in rainbow trout (Yonar et al., 2019), as well it promoted serum immunity parameters (IgM and lysozyme) in snakehead fish (*Channa argus*) (Li et al., 2022). The highest lysozyme activity was detected in rainbow trout fed curcumin supplemented diets (Kohshahi et al., 2019). Curcumin nanomicelles-enriched diets displayed higher levels of lysozyme in *L. vannamei* (Moghadam et al., 2021), and in Nile tilapia (Abdel-Tawwab et al., 2022). Furthermore, a significant increment in the lysozyme mRNA expression was observed in the Cu-NPs fed *L. vannamei* shrimp (Bhoopathy et al., 2021). Affirmative effects of *spirulina platensis* on the inherent immunity of fish, including lysozyme activity were noticed earlier (Awad et al., 2022; Cao et al., 2018a; Mohammadiazarm et al., 2021). Our finding may be accompanied by the induction of the humoral immunity of Nile tilapia after Cu-NPs and SP administration as curcumin provokes cytokines production with activating of macrophages and neutrophils, which regulate the immunity response of fish (Jagetia and Aggarwal, 2007).

A holistic view is documented between the antioxidant property and fish immunity. Antioxidative enzymes, such as SOD, CAT, and GPx protect the cellular components from oxidative stress through induce the reduction of ROS into less reactive approach (Xavier et al., 2021). The product marker of lipid peroxidation, MDA indicates the oxidative damages to the lipids. Notable increases in the activity of SOD, CAT, and GPx enzymes together with significant reduction in levels of MDA were assayed in our study, indicating to the inducing effect of antioxidant activity of Cu-NPs and SP. Higher activity of SOD and CAT enzymes coupled with lower MDA levels were documented in *L. vannamei* fed with curcumin nanoparticles additives (Bhoopathy et al., 2021; Moghadam et al., 2021). Recent study investigated the enhanced effect of antioxidant activity of Nile tilapia fed Cu-NPs (Abdel-Tawwab et al., 2022).

Comparable studies investigated the enhanced effects of *spirulina platensis* on antioxidant capacity of fish species (Awad et al., 2022; Mahmoud et al., 2018; Mohammadiazarm et al., 2021; Sayed et al., 2015; Teimouri et al., 2019). The antioxidant properties of curcumin are related with stimulating antioxidant enzymes and free radicals' removal through activating the transcription of nuclear factor erythroid 2 (Nrf2) signaling pathway (Abdel-Tawwab et al., 2022; Xu et al., 2018). Besides, polyphenols donate hydrogen or electron with capability to maintain unpaired electrons, as well as they are nitrosation reaction inhibitors, preventing oxidative deterioration via ROS scavenging and promoting the antioxidant activities (Bishayee et al., 2011; Moskaug et al., 2005). Otherwise, the chemical compounds, such as *β*-carotene, *C*-phycocyanins, vitamins, and minerals are attributed to the antioxidant property of spirulina. In specific concern, phycocyanin is a biliprotein, having the ability to modulates cyclooxygenase-2, a prostaglandin-catalyzing enzyme and so it protects against oxidative deterioration (Awad et al., 2022; Karadeniz et al., 2009).

The noteworthy lowering in the levels of glucose, total cholesterol, and triglycerides were observed in Nile tilapia fed with Cu-NPs and SP diets. *Oreochromis mossambicus* fed with curcumin enriched diets has significant lower levels of blood glucose (Sruthi et al., 2018). Many reports documented that curcumin promoted the glycogenesis with decreasing the blood glucose level in Nile tilapia (Alagawany et al., 2021; Manal, 2018). Specifically, the previously mentioned the levels biochemical indices (glucose, total cholesterol, and triglycerides) were notably diminished in Nile tilapia fed with different doses of Cu-NPs enriched diets (Abdel-Tawwab et al., 2022). The dietary addition of curcumin reduced the contents of triglyceride and total cholesterol in serum of grass carp, tilapia, and amberjack *Seriola dumerili* (Yang et al., 2022). Our current study assayed the favorable regulatory effect of curcumin on lipid metabolism, reducing the biosynthesis of plasma and hepatic cholesterol and triglyceride levels (Guan, 2015; Shin et al., 2011). Also, polyphenol compounds of spirulina species are identified as a fat reducer (Kim et al., 2013), that attributed to reduced total cholesterol and triglyceride levels in fish fed *Spirulina platensis* (Mohammadiazarm et al., 2021).

The results showed marked favorable effects of Cu-NPs and SP on the biochemical blood metabolites of Nile tilapia related to liver function (ALP, AST, ALT), renal tissue markers (creatinine and uric acid), indicating their healthy condition without liver failure or functional damage to the kidney. Including both of curcumin in gilthead sea bream (Ashry et al., 2021), and Cu-NPs in Nile tilapia diets, has resulted in similar findings (Abdel-Tawwab et al., 2022). In line with that, supplementing with *Spirulina platensis* improved the liver and renal functions in Nile tilapia (Awad et al., 2022).

In the present study, dietary Cu-NPs and SP markedly increased total protein, albumin, and globulin concentrations in the blood serum of *O. niloticus*. Substantial augmentation of serum total protein have been investigated in rainbow trout fed curcumin (Yonar et al., 2019), as well as in Nile tilapia fish supplemented curcumin (Manal, 2018), and Cu-NPs (Abdel-Tawwab et al., 2022). Also, feeding *L. vannamei* on Cu-NPs-enriched diets showed higher serum total protein and albumin levels (Moghadam et al., 2021). Moreover, Nile tilapia fed a diet containing live spirulina had higher levels of protein, albumin, and globulins (Abdel-Tawwab and Ahmad, 2009). Total serum proteins elucidate the vital index of fish's

health and nutritional status (Zahran et al., 2021). Additionally, raised serum protein and globulin levels are correlated with a potent innate immune response in fish species (Kumar et al., 2013).

Fish health is threatened by many diseases and environmental pollution that is inevitable (Taghreed, 2019). Furthermore, the stress of confinement can induce tissue damage and production losses by lowering disease resistance (Steckert et al., 2018). Similarly, in the current study, the histology of different organs of fish in the control group reported tissue alterations. On the other hand, both groups receiving Cu-NPs and SP had less severe histopathological alterations emphasizing the positive role of nutrition on disease resistance. Likewise, previous research reported that nutrition can play a major role in the incidence and severity of many fish diseases through the modulation of the immune system (Blazer, 1992).

Conclusion

The present study implied that the health promotion of Nile tilapia could be improved in respect of feeding with curcumin nanoparticles and *Spirulina platensis*. Since the results revealed marked augmentations in the growth performance, feed efficiency, immune response, and antioxidant capacity of the fish. Further, two supplements did not alter the liver and kidney tissues in Nile tilapia, which is the main farmed fresh fish of Egyptian aquaculture industry. Consequently, the use of curcumin nanoparticles and *Spirulina platensis* in proper concentration are recommended to maintain sustainability in the aquaculture of Nile tilapia.

Declarations

Data availability statement: All data generated and analyzed during this study are included in this published article.

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CRediT authorship contribution statement

Authors are equally contributed in the paper

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Figures

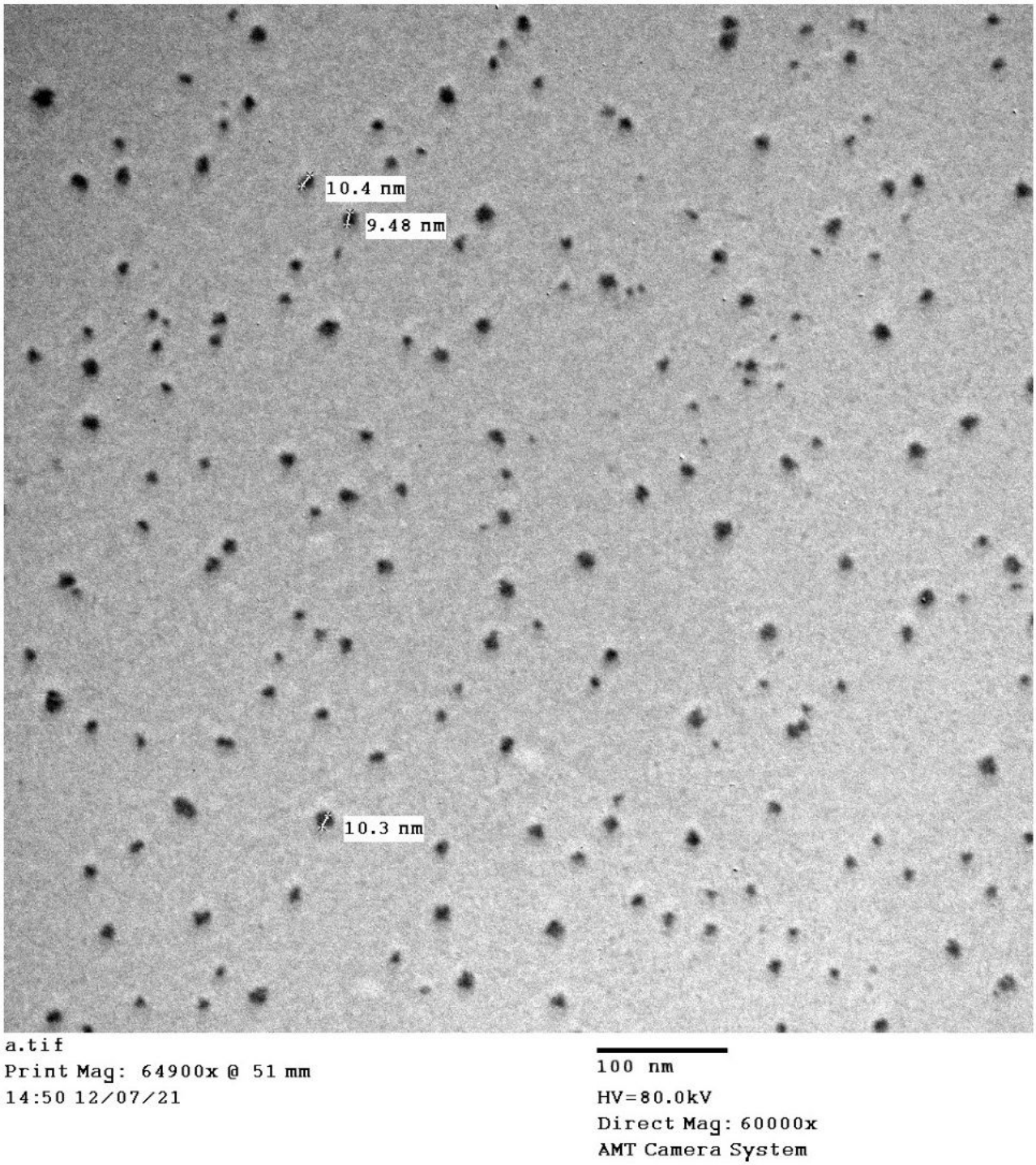

Figure 1

TEM micrograph of curcumin nanoparticles showing variable sized spherical particles with few aggregations, Bar = 82.7 ±11.1 nm.

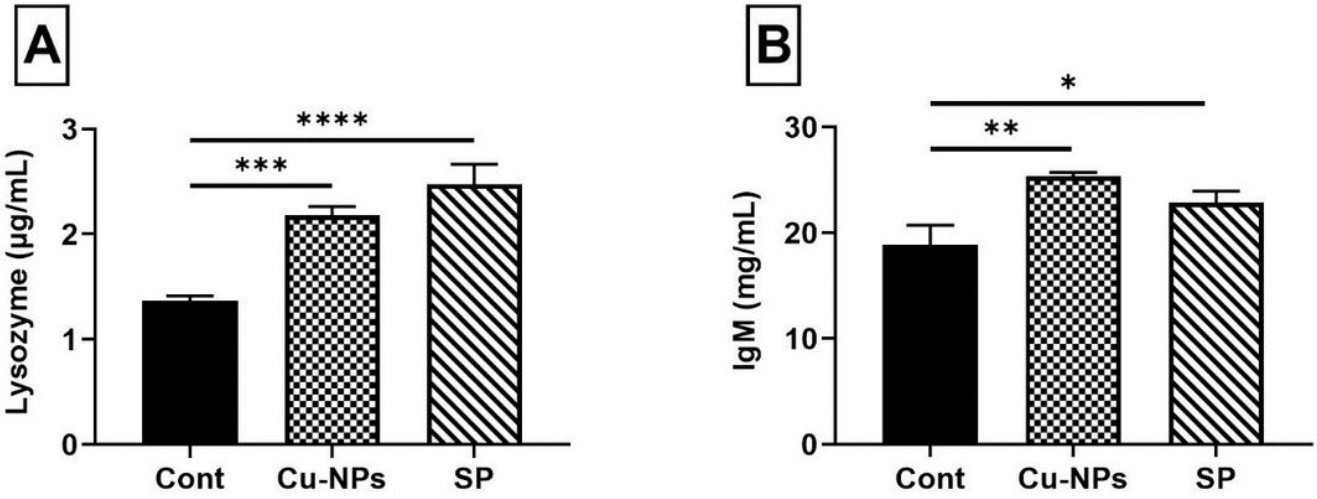

Figure 2

Effects of Cu-NPs (30 mg kg^{-1}) and SP (5 g kg^{-1}) supplementation for 56 days on serum lysozyme activity (**A**), and Ig M levels (**B**) of Nile tilapia (*O. niloticus*). Data were represented as mean ± SE (n = 5) and were analyzed by one-way ANOVA using Tukey's post hoc test. *, **, ***, **** Significant variation at $p < 0.05$, $p < 0.01$ and $p < 0.001$, $p < 0.0001$ respectively, as compared to the negative control and supplemented groups (Cu-NPs and SP).

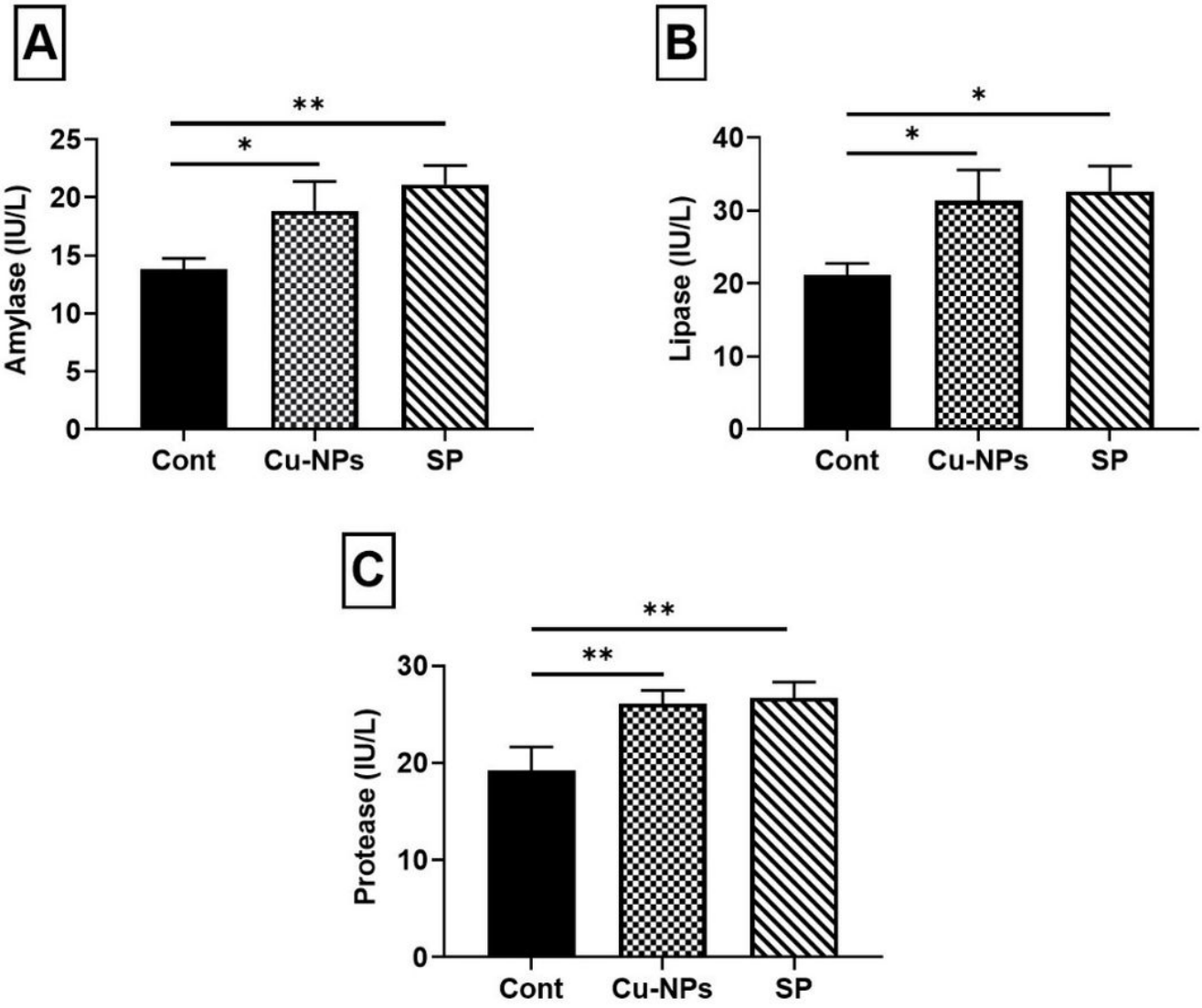

Figure 3

Impact of Cu-NPs (30 mg kg^{-1}) and SP (5 g kg^{-1}) supplementation for 56 days on the serum activities of amylase (**A**), lipase (**B**), and protease (**C**) of Nile tilapia (*O. niloticus*). Data were represented as mean ± SE (n = 5) and were analyzed by one-way ANOVA using Tukey's post hoc test. *, ** Significant variation at $p < 0.05$, and $p < 0.01$ respectively, as compared to the negative control and supplemented groups (Cu-NPs and SP).

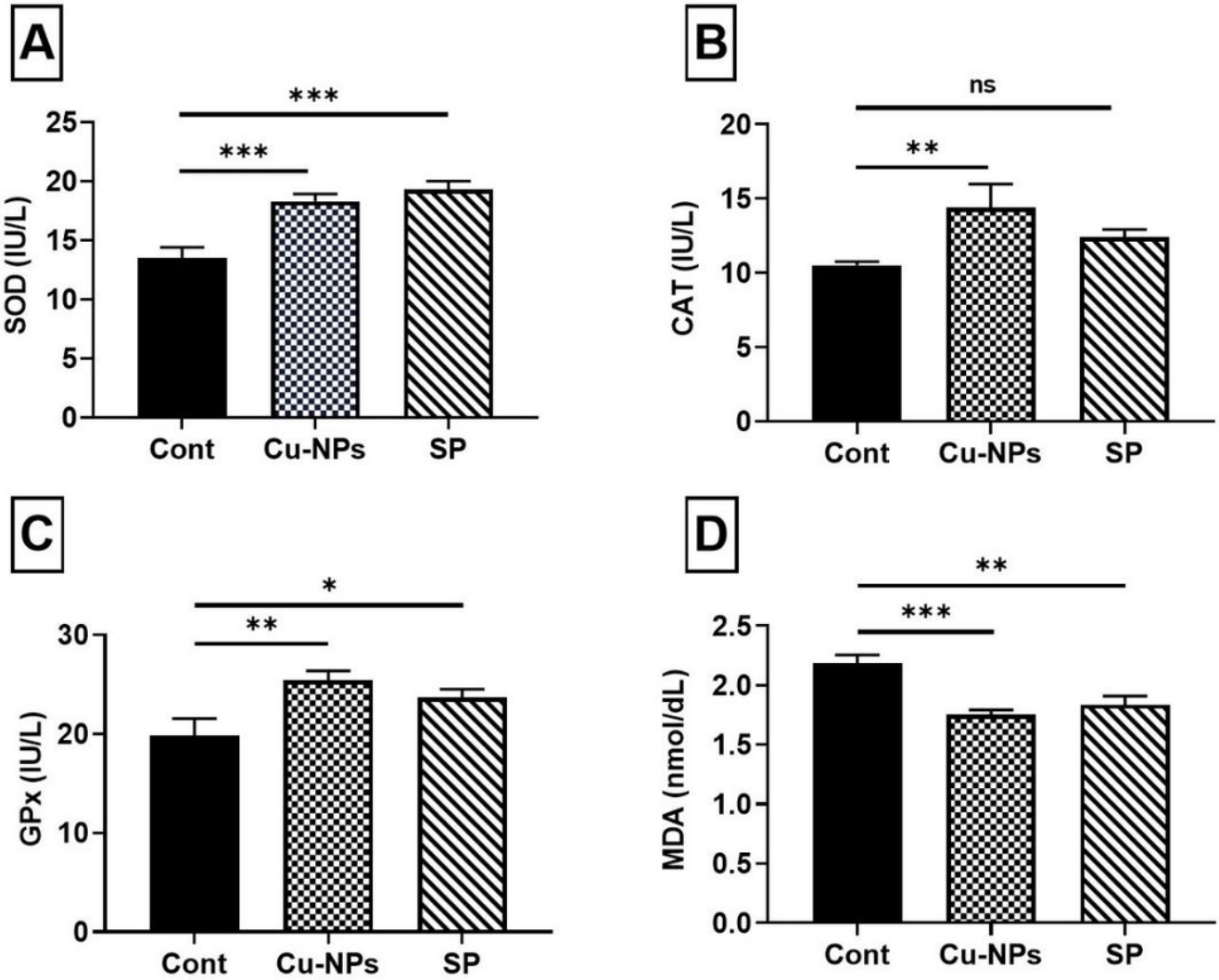

Figure 4

The activities of superoxide dismutase (SOD, **A**), catalase (CAT, **B**), glutathione peroxidase (GPx, **C**) enzymes, and malondialdehyde concentration (MDA, **D**) in the serum of Nile tilapia (*O. niloticus*) fed diets supplemented with Cu-NPs (30 mg kg^{-1}) and SP (5 g kg^{-1}) for 56 days. Data were represented as mean ± SE (n = 5). and were analyzed by one-way ANOVA using Tukey's post hoc test. *, **, *** Significant variation at $p < 0.05$, $p < 0.01$ and $p < 0.001$, as compared to the negative control and supplemented groups (Cu-NPs and SP).

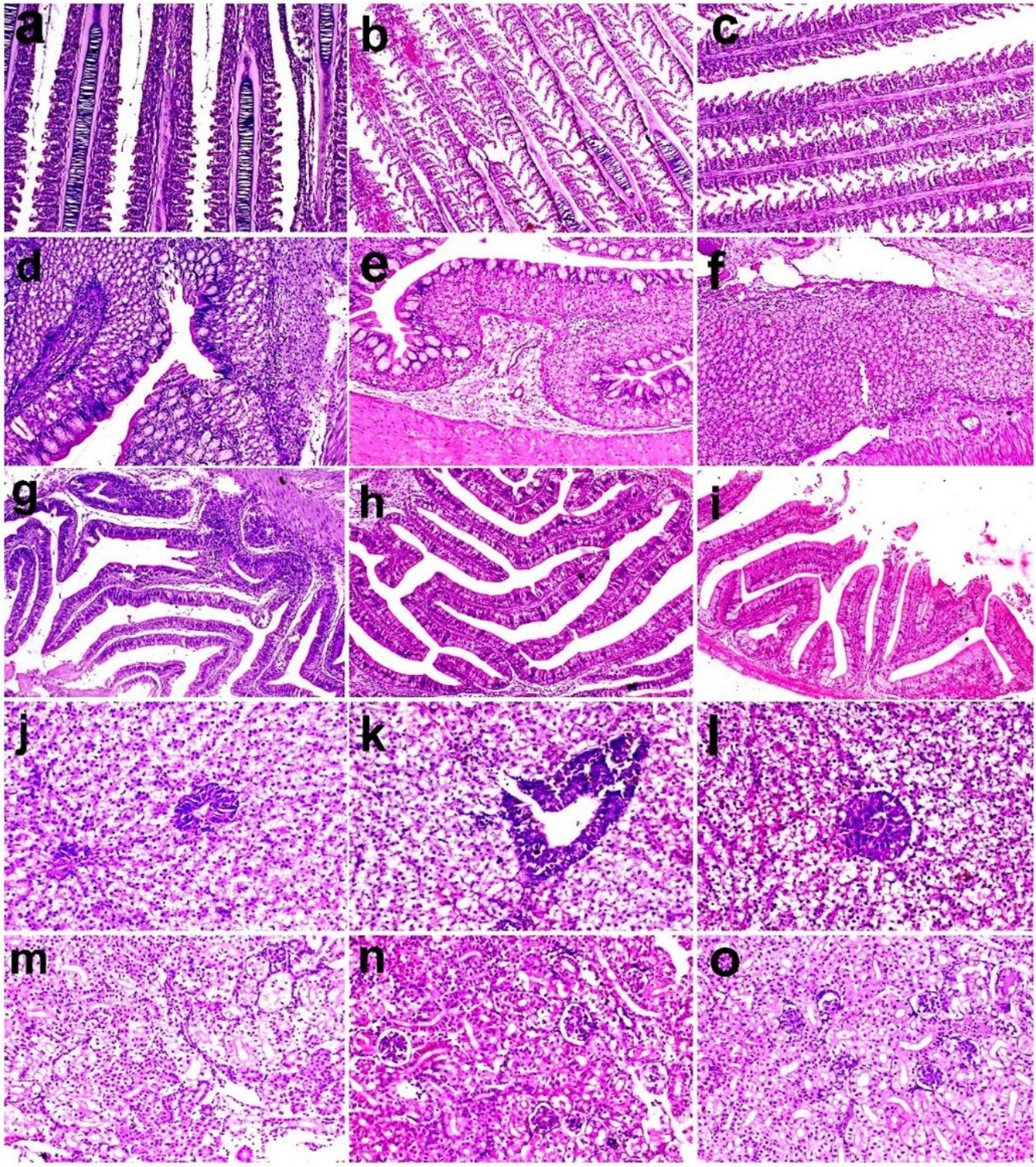

Figure 5

Histological sections of gills and internal organs of Nile tilapia, *O. niloticus*. (a) Severe diffuse hyperplastic epithelium lining secondary gill lamellae in control group, (b) moderate hyperplasia of gill epithelium in Cu-NPs, (c) and SP groups. (d) focal leukocytes infiltration and fibrosis in the gastric submucosa in the control group, (e) moderate leukocytes infiltration in the gastric submucosa in in Cu-NPs group, (f) mild histopathological alteration in the stomach in the SP group, (g) focal leukocytes

infiltration in the submucosa and focal epithelial hyperplasia in the intestine of the control group (h, i) mild histopathological alteration in Cu-NPs and SP groups. (j) mild periportal leukocytes infiltration and moderate hepatocytes vacuolation in control group. (k, l) severe diffuse hepatocytes vacuolation in Cu-NPs and SP groups. (m, n, o) mild histopathological alteration in all experimental groups (Fig. 1m, n, o).

Supplementary Files

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