Aquaculture International (2025) 33:470
https://doi.org/10.1007/s10499-025-02134-1

RESEARCH

Dietary algal zinc nanoparticles improve liver function and immunity in Nile tilapia, *Oreochromis niloticus*

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Received: 8 June 2025 / Accepted: 7 July 2025

Abstract
Green-synthesized zinc nanoparticles (ZnNP) have attracted considerable attention for use as aqua-feed supplements. The effect of ZnNPs on cellular energy, amino acid metabolism, and gene expression of major histocompatibility-II (*MHC-II*) is poorly understood. Our study explored the effect of dietary ZnNPs supplementation on hepatic function and overall health status. Farmed Nile tilapia cultured in a hapa-culturing system were fed ZnNPs with two ascending doses of 30 and 60 mg/kg dry feed for 8 weeks. Liver function enzymes, hepatic energy, amino acid profiles, gene expression analysis, and hepatic morphometry were evaluated. Non-significant changes were observed in the estimated biochemical indices, and a significant decrease ($P<0.05$) in lactate dehydrogenase (LDH) *activity* was recorded in $ZnNPs_{60}$ mg/kg dry feed compared to the control non-supplemented fish. Hepatic energy adenosine triphosphate (ATP) and CoQ10 were increased ($P<0.05$) in the supplemented groups, whereas adenosine monophosphate (AMP) showed the opposite trend. The hepatic amino acid profile indicates the selectivity of dietary ZnNPs in modulating specific protein-metabolic pathways. A higher relative expression of *IL-1β*, along with no statistical changes in *IL-10*, *HSP70*, and *MHC-II*, was recorded. Normal liver histology and a significant dose-dependent increase in mean hepatocytic area were observed. Our results suggest that incorporating ZnNPs as a functional Nile tilapia supplement is beneficial. However, further comparative investigations of different fish species are required to determine the optimal dose.

Keywords Amino acid · Liver biomarkers · Fish · Green metallic nanoparticles · Nutrition

Handling Editor: Hany Abdel-Latif

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Introduction

With the growing demand for fish as an affordable source of protein and its high nutritional value, aquaculture intensification has rapidly advanced to meet human needs. (Stevens et al. 2018; Tocher et al. 2019). However, this approach has led to significant challenges for the aquaculture industry, with disease outbreaks being among the most critical issues (Mugimba et al. 2021). Overuse of antibiotics has become a common practice (Cain 2022). Unfortunately, this has resulted in bacterial resistance, impacting the aquaculture industry and humans as final consumers (Pan et al. 2024). Coinciding with the cost of feed ingredients, fish farm owners have been forced to use poor or unconventional ingredients, which act as abiotic stressors and negatively affect fish metabolism and liver function (Aragão et al. 2022; Pan et al. 2024).

Green microalgae, including *Pediastrum boryanum* (*P. boryanum*), possess high nutritional value and are abundant in bioactive components, such as polyphenols, amino acids, and carbohydrates. These components demonstrate antioxidant and anti-inflammatory properties, as shown in our recent studies (Al-Wakeel et al. 2024a, b). Moreover, metal-chelating biomolecules in algal extracts, such as polysaccharides, peptides, and pigments, have facilitated their effective utilization in biomolecular complexes for capping metal nanoparticles (Bulgariu and Bulgariu 2020; Huq 2020). Thus, it is considered a promising microalga for bio-synthesized nanoparticles, including zinc nanoparticles (ZnNP), which are more effective than traditional zinc sources at lower dosages and indirectly help prevent environmental contamination.

ZnNP, especially those modified from biogenic sources, exhibit promising stress mitigation effects (Kumar et al. 2018, 2023). Recently, modified phytogenic ZnNPs (Mahdavi et al. 2019) and algogenic ZnNPs (Zahran et al. 2025) elicited higher antibacterial, antioxidative, and cytotoxic properties in vitro. Newly modified algogenic ZnNPs showed promising immunomodulation without harming the intestinal integrity of Nile tilapia (*Oreochromis niloticus*) (Zahran et al. 2024). Nanoparticles easily enter the body through different routes, including the oral route, which enters the intestine and then into circulation with subsequent tissues and cells, thus impacting the body (Li et al. 2022). The liver is the targeted solid organ where the detoxification process occurs and is also a primary target organ for nanoparticles regardless of the entrance route (Li et al. 2022). The liver is rich in mitochondria (Léveillé and Estall 2019). Thus, it is considered a core of intensive energy burden, where the mitochondria are the primary site of cellular energy production and the tricarboxylic acid cycle (TCA) is the main pathway responsible for energy generation (Li et al. 2022). The liver is a vital organ for amino acid homeostasis, which is essential for energy metabolism and protein synthesis (Abulikemu et al. 2023). Therefore, there is a close relationship between balanced amino acid homeostasis and the hepatic health status. Therefore, improper feed supplements have the potential to affect hepatic health in fish in different aspects, especially metabolism and immune function, leading to lipid peroxidation, inflammatory responses, and the onset of disease outbreaks. In our recent study, algogenic ZnNPs showed promising immunomodulation without harming the intestinal integrity of Nile tilapia (*Oreochromis niloticus*) (Zahran et al. 2024). However, we would like to expand our research, offering a comprehensive investigation into the biological impact of dietary ZnNPs that goes beyond previous work by integrating data across multiple physiological systems. This topic has yet to be investigated. Unlike previous studies that focused solely on toxicity or growth performance, this work uniquely demonstrates how ZnNPs influence liver enzyme activity, amino acid metabolism, immune gene expression, and energy status (ATP/AMP) simultaneously, offering new insight into their non-toxic, immunomodulatory, and metabolic regulatory roles. We aim to enhance the understanding of evaluating the safety of green ZnNPs as a functional feed supplement for Nile tilapia in realistic environmental conditions where ZnNPs would be applied practice.

Material and methods

Ethics approval statement

The experiment was conducted according to the animal use protocol approved by the Animal Care and Use Committee, Mansoura University (VM.R.23.12.134). Handling of all fish procedures and regulations followed Animal Care and Use guidelines. Furthermore, all methods were performed under the relevant policies and regulations governing the ethical use of experimental animals.

Modification of algogenic ZnNP

P. boryanum was utilized as a capping agent for the eco-friendly synthesis of ZnNP, as outlined in our recent study (Zahran et al. 2025) (Supplementary file). In the present work, bioactive compounds from *P. boryanum* microalga were extracted with magnetically treated water at 70 °C for 2 h. Gradual addition of a 1-mM solution of Zn^{2+} under magnetic stirring at room temperature for 2 h was utilized. The suspension obtained was subjected to UV irradiation for 20 min to form nanoparticles. The prepared Zn nanoparticles (ZnNPs) were filtered and kept at − 18 °C for further use. The ZnNPs were characterized extensively using UV–visible spectroscopy (PG Instruments T80 + UV/Vis spectrometer, UK), Fourier-transform infrared (FTIR) spectroscopy (Nicolet iS10 FT-IR, Thermo Scientific, USA), transmission electron microscopy (TEM) (JEOL TEM-2100, Japan), X-ray diffraction (XRD), and zeta potential analysis (Kassel, Germany). The particle size was confirmed as nearly 29.35 nm. The whole synthesis process is shown in Fig. 1. This green synthesis process is a low-cost, environmentally friendly process with minimal resources and available laboratory equipment, making it widely accessible for experimental trials in aquaculture and allied research fields.

Diet formulation

The synthesized ZnNPs were incorporated into a basal dry fish feed formulation, which was supplemented with a custom-prepared vitamin–mineral premix free of zinc. Three experimental diets were developed based on this formulation, containing 0, 30, and 60 mg ZnNPs per kg of feed, and were designated as control, ZnNPs30, and ZnNPs60, respectively. Feed ingredients were selected and balanced according to the nutritional requirements outlined by the Nrc (2011) (Supplementary Table 1). To prepare the diets, dry components were homogenized using a hand mixer (Philips HR7628, Finland), followed by the gradual addition of distilled water and sunflower oil to facilitate binding and pliability. The resulting mixtures were kneaded into a stiff dough and processed into 3-mm pellets using a meat grinder (Moulinex ME605131, France). The pellets were subsequently oven-dried at 50 °C for 24 h, sealed in plastic bags, and stored at 4 °C until use.

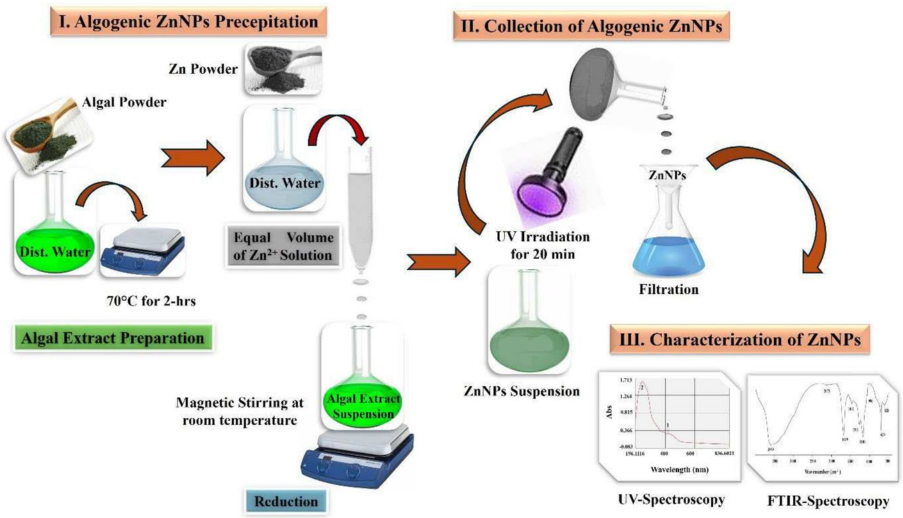

Fig. 1 A graphical representation of the modification procedures followed for the green synthesis of ZnNPs from the green microalga *Pediastrum boryanum*

Fish feeding trial

A previously established experimental design (Zahran et al. 2024) was adapted for this study. A total of 90 healthy juvenile monosex Nile tilapia (*Oreochromis niloticus*), with an average initial body weight of approximately 33–34 g, were obtained from the Fisheries Research and Application Unit, Bultéem Station Branch, Egypt. The fish were randomly allocated into three dietary treatment groups: control, $ZnNPs_{30}$, and $ZnNPs_{60}$, with each group replicated three times. Each replicate consisted of a plastic hapa (70 × 70 × 100 cm) positioned within a larger concrete pond and stocked with ten fish (i.e., 10 fish/hapa, three hapas/treatment group, totaling 30 fish/treatment). Water parameters were monitored daily and maintained within optimal aquaculture ranges: temperature 26–28 °C, dissolved oxygen 6.7–6.9 mg/L, and pH 7.0–8.0. Water was exchanged periodically following the station's standard operating procedures. Fish were hand-fed twice daily (09:00 and 15:00) at a rate of 3% of body weight (dry matter basis) under natural photoperiod and continuous aeration. The feeding trial was conducted over a period of 8 weeks.

Tissue sampling

At the end of the 8-week feeding trial, one fish was randomly selected from each replicate hapa within each treatment group, resulting in a total of nine fish ($n = 3$ per group). Selected fish were humanely euthanized using buffered MS-222 (Tricaine methanesulfonate, Finquel®, Argent) prepared at 200 mg/L tricaine and 400 mg/L sodium bicarbonate, following the protocol of Zahran et al. (2016). Blood samples were promptly collected from the caudal vasculature into plain tubes (non-heparinized), allowed to clot at room temperature for 15–20 min, and then centrifuged at 3000 rpm for 10 min. The resulting serum was separated, aliquoted, and stored at − 80 °C until used for biochemical assays to preserve enzymatic activity.

Post-euthanasia, fish were dissected to collect the liver and anterior kidney. Liver tissues were fixed in 10% neutral buffered formalin for histomorphometric assessment. Additional liver portions were homogenized in phosphate-buffered saline (PBS, pH 7.4) under cold conditions (4 °C) and centrifuged at 1700 × g for 15 min. The supernatants were aliquoted and stored at − 80 °C for later analysis of antioxidant markers. Approximately 50–100 mg of anterior kidney tissue was placed in RNAlater solution (Invitrogen, USA) and stored at − 80 °C for subsequent transcriptomic analysis.

Biochemical indices of blood

Serum levels of key hepatic health biomarkers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), were measured to evaluate liver function. Biochemical assays were conducted using Cobas reagent kits on the COBAS INTEGRA® 400 Plus automated analyzer (Roche Diagnostics, Indianapolis, IN, USA). All procedures were carried out in accordance with the manufacturer's established protocols.

Determination of hepatic energy biomarkers and CoQ10

The concentrations of adenosine nucleotides, ATP, ADP, and AMP, in liver tissue were quantified using a modified high-performance liquid chromatography (HPLC) method based on Teerlink et al. (1993) Approximately, 500 mg of liver tissue was homogenized in 5 mL of ice-cold 10% potassium chloride solution, followed by centrifugation at 5000 rpm for 20 min at 4 °C to obtain a clear supernatant. For deproteinization, 200 µL of the supernatant was combined with 1 mL of 70% methanol. The resulting samples were prepared for analysis on a Nova-Pak™ C18 column. Chromatographic separation was carried out using an isocratic mobile phase consisting of 0.1 M KH_2PO_4 (pH 6.0) and methanol, delivered at a flow rate of 1.0 mL/min. The injection volume was 20 µL, and detection was performed at 254 nm using the ChemStation software package. For the determination of Coenzyme Q10 (CoQ10), a gradient elution protocol was applied, using ethanol as mobile phase A and a 50:50 (v/v) mixture of 2-propanol and acetonitrile as mobile phase B, based on the method of Niklowitz, et al. (2013). Coenzyme Q9 (CoQ9) was used as an internal standard to assess recovery and compensate for any sample loss during extraction and processing. Calibration curves for both the nucleotides and CoQ10 were prepared using certified reference standards and demonstrated high linearity ($R^2 > 0.998$). The limits of detection (LOD) and quantification (LOQ) were determined using signal-to-noise ratios of 3:1 and 10:1, respectively.

Hepatic amino acid profile

Liver free amino acid profiles were determined using the high-performance liquid chromatography (HPLC) following phenylisothiocyanate (PITC) derivatization, following the method of Heinrikson and Meredith (1984). The analysis was performed on an Agilent HPLC system equipped with a quaternary pump, thermostated column compartment, Rheodyne injector (20-µL loop), and UV detector, with data acquisition and processing carried out using the ChemStation software. A PICO-TAG column (Waters, USA) was utilized, along with dedicated elution buffers and amino acid standards supplied by Waters. Tissue samples were first homogenized in 75% aqueous HPLC-grade methanol. To derivatize the amino acids, the dried extract was treated with a mixture containing methanol, 1 M sodium acetate trihydrate, and triethylamine (TEA), followed by reaction with the PITC reagent. The derivatized samples were reconstituted in disodium hydrogen phosphate buffer (pH 7.4) containing 5% acetonitrile. A 20-μL volume of each sample or standard was injected for analysis. Norleucine was included as an internal standard for derivatization efficiency and recovery variation. Quantification of individual amino acids was based on external calibration curves, which demonstrated excellent linearity ($R^2 > 0.997$). The method's sensitivity was confirmed with limits of detection (LOD) and quantification (LOQ) ranging from 0.1 to 0.3 μg/mL, depending on the analyte.

Gene transcription analysis of head kidney

Total RNA was extracted from approximately 100 mg of anterior kidney tissue using Genzol™ reagent (Geneaid Biotech Ltd., Taiwan), following manual homogenization and without DNase treatment. The RNA pellet was resuspended in TE buffer (pH 8.0), following the protocol described by Gorgoglione, et al. (2016). RNA quantity and purity were assessed using a Nanodrop spectrophotometer (Q5000, Quawell, Massachusetts, USA). First-strand cDNA was synthesized from 1 μg of total RNA using the TOPscript™ RT DryMIX (dT18) cDNA Synthesis Kit (Enzynomics Co., Daejeon, Republic of Korea), following the manufacturer's recommendations. Quantitative real-time PCR (RT-qPCR) was conducted on a QuantStudio1™ Real-Time PCR System (Applied Biosystems™, Thermo Fisher Scientific, USA) to determine the relative transcript levels of *interleukin-1β (IL1-β)*, *IL-10*, *heat shock protein 70 (HSP70)*, and *major histocompatibility complex class II (MHC-II)*. The housekeeping gene *β-actin* was used as an internal reference. Primers for the target and reference genes were adopted from previously validated studies (Elbahnaswy and Elshopakey 2020; Zahran et al. 2021). Amplifications were carried out using Solg™ 2X Real-Time PCR Smart mix (SolGent Co., Daejeon, Korea) in 20-μL reactions, performed in technical triplicates. The thermal profile included an initial denaturation at 95 °C for 1 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. A melting curve analysis was performed post-amplification to verify product specificity. Relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

Hepatic integrity and biometry evaluations

Liver tissues were preserved in 10% neutral-buffered formalin, followed by routine processing that included dehydration, paraffin embedding, and microtome sectioning at a thickness of 4–5 μm. The sections were stained with hematoxylin (5 min) and eosin (2 min) for histological evaluation, then dehydrated, cleared, and mounted for microscopic analysis, according to the standard protocol described by Bancroft and Gamble (2008). The hepatocyte area on uniformly prepared sections was assessed using ImageJ software to ensure consistency. The hepatocytic area of the sampled liver tissue was estimated as the mean area (μm^2) of 100 hepatocytes/fish, as described by (Picoli et al. 2019). Initially, H&E-stained sections of the liver were randomly scanned at 40× magnification under a light microscope (Olympus CX 31). Then, five randomly selected replicate micrograph fields per sampled fish were captured using a connected digital camera (Olympus DP 21) (i.e., 5 micrographs × 9 sampled fish = 45 micrographs). Subsequently, the area of each captured hepatocyte of all the sampled fish during our experiment (i.e., 900 hepatocytes) was measured on the micrographs, and the mean hepatocytic area was then calculated for each supplemented group. All data were calculated using the ImageJ software (version 1.41o, Public Domain, BSD-2, https://imagej.net/Ops) for histogram display.

Statistical analysis

Data were initially assessed for normal distribution and homogeneity of variance using the Kolmogorov–Smirnov and Levene's tests, respectively. One-way analysis of variance (ANOVA) was then performed to evaluate differences among treatment groups, using the GraphPad Prism® software (version 8.4.2; GraphPad Software, Inc., USA). Hepatic energy-related biomarkers and CoQ10 concentrations were visualized using box plots, while ridgeline plots were employed to depict the distribution of free amino acid profiles, both generated using the R statistical software. Statistical significance was determined at $P < 0.05$, with values of $P < 0.01$ and $P < 0.001$ considered highly significant. All results are presented as mean ± standard error (SE).

Results

Blood biochemical indices

No statistically significant differences ($P > 0.05$) were observed between the farm groups in the levels of ALT, AST, and ALP serum enzymes (Fig. 2). Their levels recorded a non-significant fluctuation ($P > 0.05$) in both the algogenic ZnNPs (30% and 60%)-supplemented groups compared to the control non-supplemented group. On the other hand, a diminution with no significant difference ($P > 0.05$) was recorded in fish supplemented with 30% algogenic ZnNPs. In comparison, a significant decrease ($P < 0.05$) in LDH levels was recorded in the 60% algogenic ZnNP-supplemented group (Fig. 2).

Hepatic energy biomarkers and CoQ10

ATP levels increased ($P < 0.05$) in $ZnNPs_{60}$ compared to those in the control, suggesting enhanced energy generation within hepatic cells. Conversely, AMP showed an opposite trend. However, ADP concentrations did not differ among the groups ($P > 0.05$). Both supplemented groups exhibited higher CoQ10 levels ($P < 0.05$), demonstrating the role of ZnNPs in supporting mitochondrial functionality and energy production. Figure 3A, B, C, and D shows box plots representing the data for ATP, ADP, AMP, and CoQ10.

Amino acids profile

The ridgeline plot (Fig. 4) illustrates the effects of ZnNPs supplementation on the 16 amino acids identified in the liver homogenate. Certain amino acids, including isoleucine (ISO), leucine (LEU), tyrosine (TYR), valine (VAL), cysteine (CYS), alanine (ALA), threonine (THR), and serine (SER) (Fig. 4A), remained consistent across the supplemented groups, with no statistically significant variations ($P > 0.05$). Conversely,

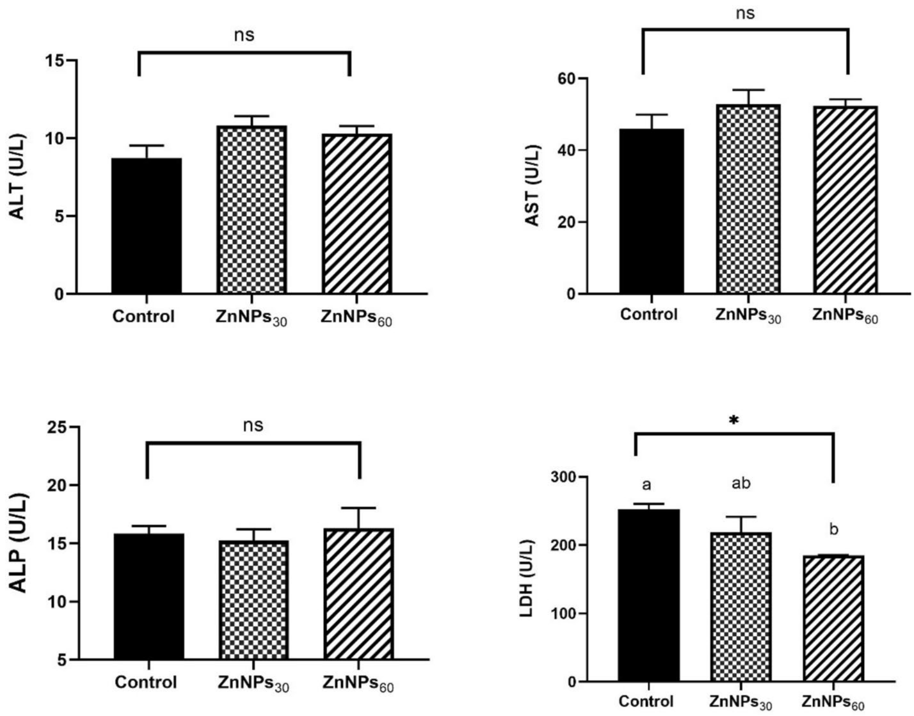

Fig. 2 Activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) enzymes in sera of farmed *O. niloticus* fed diets supplemented with algogenic ZnNPs (30 or 60 mg/kg dry feed) or non-supplemented diets for 8 weeks. Values are presented as mean ± SE. Different letters indicate a significant difference between groups (ANOVA with post hoc Tukey test), and the level of significance is indicated by an asterisk as follows (*$P < 0.05$, **$P < 0.001$, and ***$P < 0.0001$)

both ZnNPs and ZnNP-supplemented groups, particularly the $ZnNPs_{60}$ group, exhibited significant increases ($P < 0.05$) in glycine (GLY), arginine (ARG), proline (PRO), and methionine (METH) levels (Fig. 4B). The opposite ($P < 0.05$) was observed for aspartic acid (ASP), glutamic acid (GLU), histidine (HIS), and lysine (LYS) levels (Fig. 4C).

Gene expression

Feed supplementation with algogenic ZnNPs revealed changes in the relative expression of the genes targeted in our study in different ways (Fig. 5). A significant upregulation ($P < 0.001$) was observed in the *IL-1β* mRNA level (~ fourfold change in $ZnNPs_{30}$ and ~ fivefold change in $ZnNPs_{60}$-supplemented groups compared to the control group), with the highest expression recorded in the $ZnNPs_{60}$ group. Notably, a significant increase ($P < 0.05$) ~ twofold change in the *IL-1β* mRNA level was observed upon increasing the supplemented dose of ZnNPs. As for the stress, inflammatory, and anti-inflammatory genes, *HSP70*, *MHC II*, and *IL-10*, non-significant ($P > 0.05$) changes were observed in their expression levels among the groups.

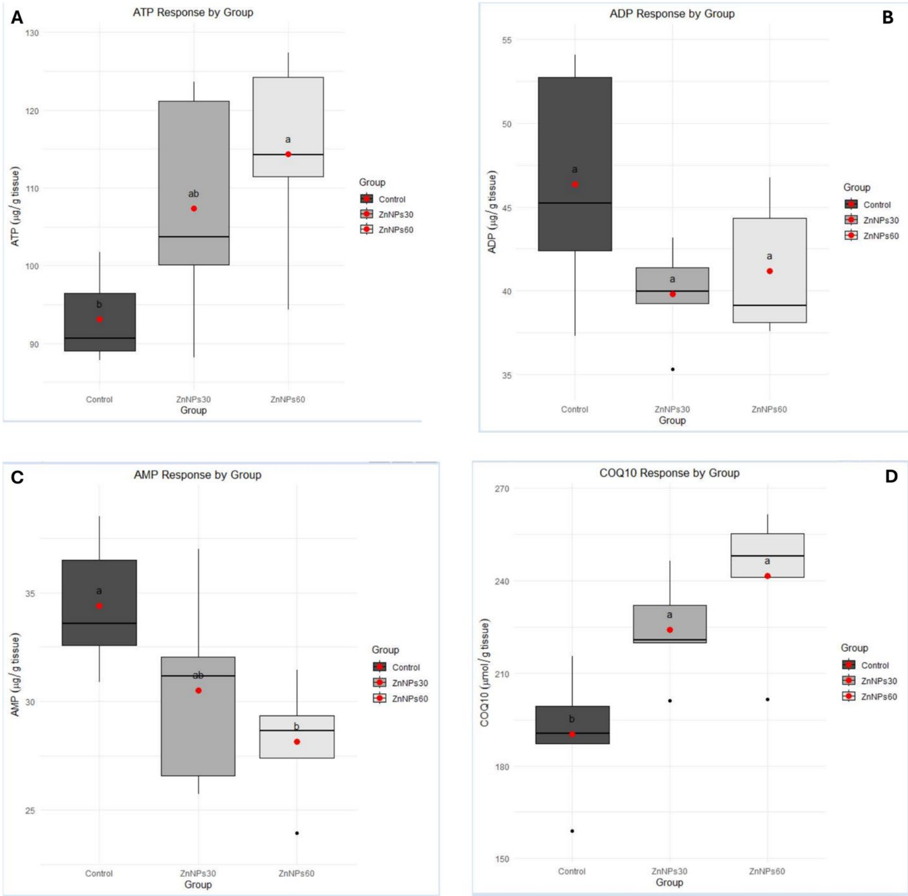

Fig. 3 **A, B, C** Hepatic energy biomarkers, and **D** CoQ10 in the liver homogenate of farmed *O. niloticus* fed diets supplemented with algogenic ZnNPs (30 or 60 mg/kg dry feed) or non-supplemented diets for 8 weeks. Values are presented as mean ± SE. Groups are statistically significant at $P < 0.05$ (ANOVA with post hoc Tukey test)

Hepatic integrity and biometry evaluations

Hepatic histological examination (Fig. 6A) of Nile tilapia showed that liver architecture was preserved in all groups, with clearly defined hepatocytes (H), sinusoids (S), and central veins (CV), and no signs of inflammation, necrosis, or vacuolation. The hepatocytes appeared polygonal with centrally located nuclei, and no histopathological alterations were observed in either the control or ZnNPs-treated groups. Dietary supplementation with algogenic ZnNPs was shown to augment the mean area of hepatocytes of farmed fish compared to non-supplemented fish. The data indicated a statistically significant augmentation of the hepatocytic mean area with increasing ZnNPs concentrations in the fish feed. Compared to the non-supplemented group, a significantly ($P < 0.05$) larger mean area was recorded in the $ZnNPs_{60}$ group. In contrast, no statistically significant difference ($P > 0.05$) was observed between the ZnNPs30 group and supplemented groups (Fig. 6B).

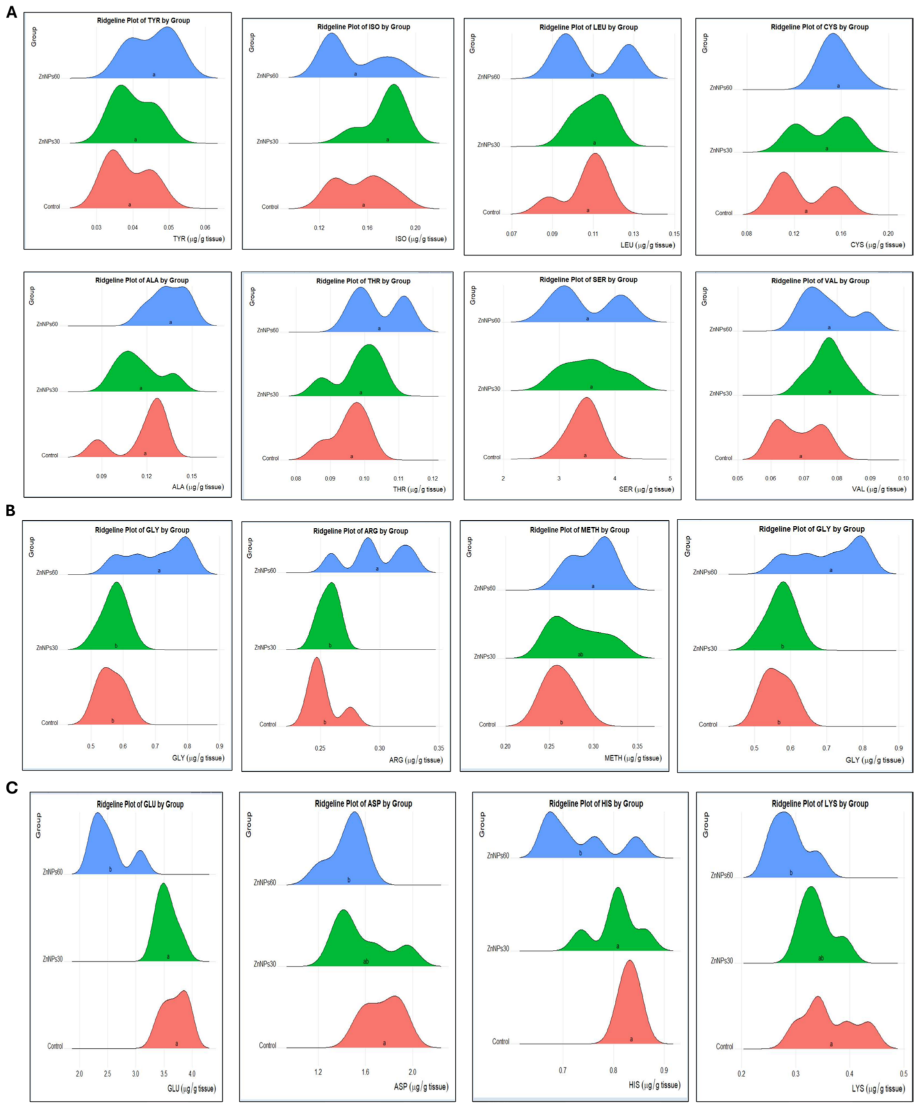

Fig. 4 Ridgeline plot mapping the levels of 16 amino acids targeted in the liver homogenate of *O. niloticus* supplemented or non-supplemented with ZnNPs with two doses of 30 or 60 mg/kg dry feed. **A** Isoleucine (ISO), leucine (LEU), tyrosine (TYR), valine (VAL), cysteine (CYS), alanine (ALA), threonine (THR), and serine (SER). **B** Glycine (GLY), arginine (ARG), proline (PRO), and methionine (METH). **C)** Aspartic acid (ASP), glutamic acid (GLU), histidine (HIS), and lysine (LYS)

Discussions

As the liver is commonly regarded as a significant organ for the deposition of NPs after absorption, it is important to ensure the safety of the introduced dietary nanostructure supplement at the organismal level. Stress causes tissue degeneration in hepatic cells and the central vein, changes the relative expression of key metabolic genes in the liver, and may

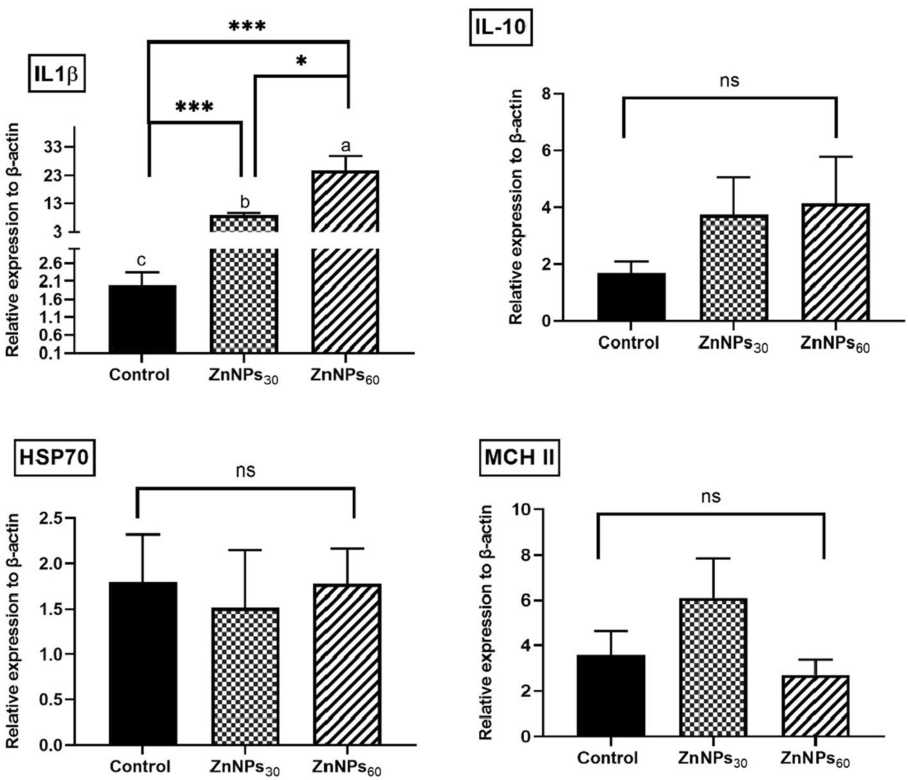

Fig. 5 The expressions of IL-1β, IL-10, HSP70, and MHC II mRNA levels relative to β-actin housekeeping gene of farmed *O. niloticus* fed diets supplemented with algogenic ZnNPs (30 or 60 mg/kg dry feed) or non-supplemented diets for 8 weeks. Values are presented as mean ± SE, and the statistically significant differences are displayed as letters on bars. Asterisks indicate significant levels $*P<0.05$, $**P<0.01$, and $***P<0.001$ (ANOVA with post hoc Tukey test)

result in mitochondrial bioenergetic dysfunction and hepatotoxicity (Kumar et al. 2012; Liu et al. 2016; Qian and Xue 2016; Santos et al. 2012); Sun et al. 2019). In the present study, no alterations were observed in the liver function enzymes with higher LDH activity. AST and ALT are non-specific enzymes in the blood that exist in various organs, including the liver. The increase in their activities could be due to organ damage, or their decline could be due to reduced production or excretion (Gharaei et al. 2020). The unchanged activity of ALP may indicate that its regulation was normal. Additionally, it has been reported that increased levels of LDH in the blood are linked to tissue damage; therefore, reducing their activity suggests improvement in cellular function (Gharaei et al. 2011). These findings indicate that ZnNPs do not affect liver function or overall metabolic balance, indicating a healthy physiological state (Liu et al. 2014). Our results are consistent with those of previous studies on Nile tilapia and beluga (*Huso huso*) fed curcumin-assisted-synthesized ZnO nanoparticles (Gharaei et al. 2020; Yazdani et al. 2023).

AMP and ATP are the real energy currencies crucial to cell metabolic processes (Sikkema et al. 2019). Because of the body's energy sensing, elevated levels of ATP minimize the cellular need for the adenylate kinase reaction for the regeneration of ATP from ADP

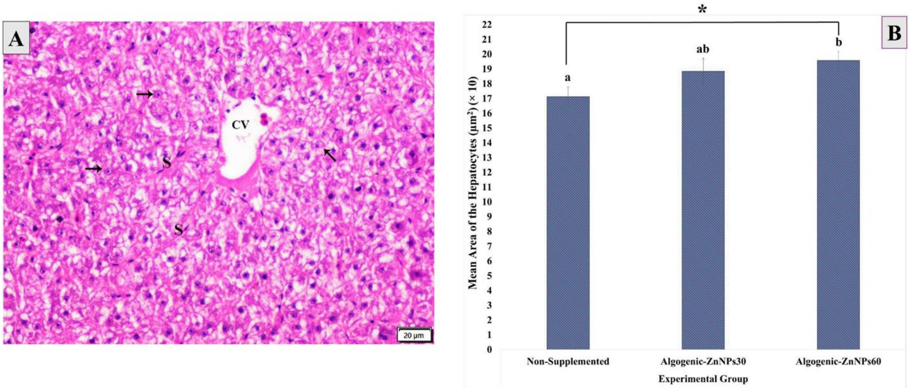

Fig. 6 Hepatocytic integrity and biometry of farmed *O. niloticus* fed diets supplemented with algogenic ZnNPs (30 or 60 mg/kg dry feed) or non-supplemented diets for 8 weeks. **A** Photomicrograph of the liver displays the normal hepatic tissue of a representative section of the liver (H&E; 20 µm bar); (CV) normal central vein, (arrows) normal hepatocytes with eosinophilic cytoplasm and vesicular nucleus, and sinusoids (S) tunneled by hepatocytes to form two or more cell-thick plates. **B** A histogram displays the Mean hepatocytic area of each experimental group. Values are presented as mean ± SE. Statistically significant differences ($P < 0.05$) are displayed as different letters on bars. Post hoc Tukey test by ANOVA is indicated by asterisks (*$P < 0.05$, **$P < 0.001$, and ***$P < 0.0001$)

and AMP. As evidenced in this study, high ATP levels are associated with low AMP levels to maintain the cellular homeostatic network (Dzeja and Terzic 2009). In an antagonistic way, CoQ10 is an endogenous antioxidant and a key factor in the mitochondrial respiratory chain (Duberley et al. 2014). Our data showed higher CoQ10 levels in both the supplemented groups. This coenzyme can modulate metabolic pathways inside or outside the mitochondria and may contribute to overcoming several metabolic diseases (Hidalgo-Gutiérrez et al. 2021). It plays a critical role in cellular energy production by assessing mitochondrial mass production and supporting its function in energy production as it acts as a carrier for electron transport during the mitochondrial respiratory chain for ATP synthesis (Noh et al. 2013). Moreover, CoQ10 elicits antioxidant effects, and its deficiency is associated with mitochondrial oxidative stress (Silva et al. 2022). Recently, supplementation of the Nile tilapia diet with CoQ10 was reported to improve growth and health and augment disease resistance against infections (Abbas et al. 2020; El Basuini et al. 2021, 2020). Zn maintains mitochondrial homeostasis by controlling protein synthesis, and its deficiency decreases ATP production inside cells (Elmetwalli et al. 2022; Lee 2018). As evidenced in the current study, increased ARG, METH, and GLY levels can promote mitochondrial biosynthesis, augment the antioxidant response, and are indirectly implicated in inflammatory suppression. Altogether, it can be inferred that dietary ZnNPs at both doses (30 and 60 mg/kg) protect mitochondrial integrity from oxidative damage and support energy production, which reduces oxidative stress in the culture system.

Our results revealed that certain amino acids were positively or negatively affected by ZnNPs supplementation, while the others did not show any changes. Our findings corroborate the selectivity of ZnNPs in modulating certain protein-metabolic pathways and hence prove the potential of ZnNP supplementation to influence amino acid metabolism in a targeted way. In this context, elevated levels of GLY, ARG, PRO, and METH amino acids suggest that zinc acts as a cofactor for the activity of several enzymes involved in amino acid metabolism, which are involved in the synthesis of several structural proteins important for liver function and repair, such as collagen and elastin (de Paz-Lugo et al. 2018; Gaar et al. 2020). ZnNP supplementation enhances protein synthesis, which treats metabolic syndrome (Olechnowicz et al. 2018). In addition, Zn is an essential element for several proteins that mediate oxidative stress defense and DNA repair (Song et al. 2009). Moreover, dietary zinc activates enzymes related to the urea cycle and the trans-sulfuration pathway, which are crucial for the actual metabolic function of sulfur-containing amino acids, including METH amino acid (Sansuwan et al. 2023). Therefore, the increased levels of GLY, ARG, PRO, and METH amino acids in the liver homogenate in our study reflect the role of Zn in their metabolic pathways upon ZnNPs dietary supplementation. The opposite trend was seen with ASP, GLU, HIS, and LYS amino acids, which reflect their altered metabolic pathways by ZnNPs supplementation. This finding could be attributed to an increase in ATP production (see below), which induces higher utilization of amino acids in the tricarboxylic acid (TCA) cycle and controls some enzymes responsible for the conversion of these amino acids into other metabolites (Hojyo and Fukada 2016). Moreover, given that Zn enhances immune functions, the utilization of HIS and LYS increases, as they are vital for immune responses and serve as precursors for various biological functions (Khan et al. 2020).

It is noteworthy that ZnNPs are incorporated into the homeostatic regulation of the body by maintaining the levels of key metabolic amino acids (ISO, LEU, TYR, VAL, CYS, ALA, THR, and SER) to ensure metabolic homeostasis. This may indicate that the metabolism of these stable proteins is tightly regulated and less influenced by ZnNPs supplementation, or that they are synthesized from precursors that do not significantly interact with zinc-dependent enzymes. This also aligns with our findings of normal levels of ALT and AST, suggesting improved metabolic function and highlighting the selective modulatory effects of dietary ZnNPs on amino acid metabolism. This effect is due to the role of zinc in activating the enzymes that regulate the synthesis and degradation of amino acids (Olechnowicz et al. 2018). However, the effect of ZnNPs on amino acid concentration could be dose- or duration-dependent and is influenced by the metabolic state of the liver, which requires further investigation.

A dose-dependent increase was observed in the relative expression of the proinflammatory *IL-1β* cytokine gene, with the highest expression recorded in the $ZnNPs_{60}$ group. Immunomodulation of fish by feed supplementation has previously been observed. In line with the current findings, in our most recent study (Zahran et al. 2024), dietary BIO-ZnNPs (40 and 60 mg/kg dry feed) were found to boost Nile tilapia immunity and induce the expression level of the *IL-1β* in a dose-dependent manner (Diab et al. 2022). Similarly, with the same doses of dietary ZnONPs (30 and 60 mg/kg dry feed), upregulation of the relative expression of *IL-8* and *IL-1β* cytokine genes in Nile tilapia was reported after 60 and 120 days, with higher values at higher doses (Awad et al. 2019; Tawfik et al. 2017). This might be evidence of the role of Zn in the promotion of cytokine signaling molecules for immune response modulation upon administration of high doses of ZnNPs. On the other hand, *IL-10*, *HSP70*, and *MHC II* expression levels did not display any statistically significant changes, suggesting that ZnNPs supplementation elicited no stress or inflammatory response. As an anti-inflammatory cytokine, *IL-10* plays a crucial role in reducing inflammation and inhibiting unnecessary T-cell responses to microbial infections. This action helps prevent tissue damage and the development of chronic inflammatory conditions (Li and Flavell 2008). The lower induction of the *HSP70* gene indicates less stress metabolism and higher welfare of the supplemented fish groups. *HSP70* is a member of the heat shock protein family, which is induced in response to several stress conditions and metabolic interactions (El-Leithy et al. 2019; Jesus et al. 2013; Place and Hofmann 2005; Yamashita et al. 2010). Similarly, Muralisankar et al. (2014) suggested that dietary ZnNPs not exceeding 60 mg/kg can be introduced to *Macrobrachium rosenbergii* for regular survival, growth, and immunity. Our data could be attributed to the antioxidant role of Zn, which is related to its ability to scavenge free radicals (Zahran et al. 2025). In addition, it is involved as a cofactor for copper-zinc superoxide dismutase (Cu, Zn-SOD), a primary antioxidant enzyme in the body. Furthermore, our results of the increased levels of METH and glycine support the indirect enhancement of GSH, where these amino acids contribute by supporting pathways that enhance cysteine availability or the overall amino acid balance necessary for optimal GSH production (Marreiro et al. 2017). In contrast, the *MHC* gene family presents the peptide antigens to T lymphocytes, which initiates an adaptive immune response against invaders, and their relative expression is induced by pathogenic infestation (Monzón-Argüello et al. 2014; Yamaguchi and Dijkstra 2019). Our data emphasize the role of Zn in suppressing inflammation, which is either due to the inhibition of the NF-κB signaling pathway, as evidenced in our recent study (Zahran et al. 2024), or through the activation of the Nrf2/HO-1 signaling pathway, which reduces the production of proinflammatory cytokines and promotes the production of immunoglobulins (Al-Gheffari et al. 2024; Alsulami and El-Saadony 2024; Zahran et al. 2024). Taken together, our data corroborated other parameters analyzed herein and reflected the histological observations, suggesting that ZnNPs supplementation in this study was safe for Nile tilapia without triggering any inflammatory condition.

Our data showed a normal histological arrangement with abundant hepatocytes (H), prominent nuclei, and sinusoids. Given that the liver is recognized as one of the organs with plenty of mitochondria, reflecting its vital role in metabolic processes, our data showed that it promoted mitochondrial functionality, antioxidative capacity, and protein metabolism alongside the role of Zn in stabilizing the membrane integrity and preventing enzyme leakage. Collectively, these findings contributed to the normal hepatic morphometry. Although no adverse effects were observed in liver histology or stress gene expression, this study did not quantify zinc accumulation in liver tissue. However, our previous work (Zahran et al. 2024) showed that Zn content was within the safe range in freshwater muscle following the same ZnNPs dose supplementation, which is important concerning humans as final consumers. Our data are consistent with those of previous studies on Nile tilapia-fed phyco-synthesized ZnO-NPs at 40 or 60 mg/kg (Diab et al. 2022), Nile tilapia-fed bacterial ZnNPs (Alsulami and El-Saadony 2024), and ZnO-NPs (Kurian and Elumalai 2021) at 100 mg/kg.

While this study provides important insights into the immunometabolic and hepatic responses of Nile tilapia to dietary zinc nanoparticles (ZnNPs), certain limitations should be acknowledged. Although the 8-week feeding duration offers valuable information on subchronic effects, further studies are needed to explore longer-term impacts, particularly concerning reproductive performance, tissue Zn accumulation, and potential residue concerns for food safety under commercial farming conditions to support their safe and effective application in aquaculture.

Conclusions

The present study is the first to investigate the beneficial effects of green-synthesized ZnNPs on the liver health biomarkers of Nile tilapia. The findings show improvements in liver enzyme functions, enhanced mitochondrial activity through increased energy biomarkers, better amino acid metabolism, immune response regulation, and reduced inflammatory mediators, while maintaining normal liver structure. Biosynthesized ZnNPs, created using a cost-effective and eco-friendly algal extract method, present a promising alternative to traditional zinc sources. However, larger scale future studies are necessary to confirm these effects. These results support the potential use of ZnNPs as functional feed additives in fish farming.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10499-025-02134-1.

Acknowledgements This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Grant No. KFU252145].

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Data availability Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval The experiment was conducted according to the animal use protocol approved by the Mansoura University Animal Care and Use Committee (VM.R.23.12.134). All fish handling procedures and regulations followed Animal Care and Use guidelines. Furthermore, all relevant organizational and government rules and regulations governing the ethical use of the experimental animals were followed.

Competing interests The authors declare no competing interests.

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