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Comparative effects of dietary zinc forms on performance, immunity, and oxidative stress-related gene expression in Nile tilapia, *Oreochromis niloticus*

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ABSTRACT

Zinc is one of the essential elements, which plays an important role for fish performance. It occurs naturally in different types of inorganic, nano-sized, organic, and elemental forms. Thus, the present study was carried out to assess the effects of dietary Zn forms on the performance of Nile tilapia, *Oreochromis niloticus* (L.). Three iso-nitrogenous (30% crude protein) and isolipidic (7.0% lipids) diets were formulated containing biogenic zinc oxide nanoparticles (ZnO-NP), ZnO supported on kaolinite (ZnO-K), or mineral Zn form ($ZnSO_4$) as a control diet. Fish (2.0–2.5 g) were fed on one of the tested diets in triplicates up to apparent satiation four times a day for 12 weeks. Results showed that the highest growth performance and feed intake were recorded in fish fed ZnO-K. However, this diet elevated villus width/length, absorption area, and goblet cells and significantly enhanced activities of intestinal digestive enzyme and cholecystokinin content. Comparing to the control diet, serum alanine and aspartate aminotransferases were significantly lower, while serum total protein, albumin, and globulin contents were obviously higher in fish fed ZnO-K containing diets. Additionally, significant higher values of total antioxidant capacity, catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activity accompanied with lower value of malondialdehyde (MDA) were observed in fish fed ZnO-K in diets. The expression of SOD, CAT, and IgM-2 genes were significantly up-regulated in the liver of fish fed a diet containing ZnO-K compared with other Zn forms. Results obtained herein indicated that the ZnO-K was the optimum Zn form followed by biogenic ZnO-NP to enhance growth performance, health, immunity, and oxidative stress biomarkers of Nile tilapia.
Statement: The authors stat that their individual contribution is equal.

1. Introduction

Fish as other animals require different micronutrients, which play an important role for their survival, growth, health, and reproduction (Aliko et al., 2018). Zinc (Zn) is one of these microelements used as a specific cofactor of many metabolic pathways, growth, bone mineralization, and physiological functions in fish (Eckerich et al., 2001; Eide, 2006; Maret and Krężel, 2007; Liang et al., 2012). Freshwater fish have the potentiality to absorb Zn from both food and water, but the dietary Zn is the primary source for their absorption where Zn level in freshwater is inadequate to meet their requirements (Willis and Sunda, 1984; Spry et al., 1988). So, it should be added to fish diets to meet fish requirements to maintain their health and high growth (Shearer et al., 1992). Dietary Zn requirements have been established for many fish species by using zinc sulfate ($ZnSO_4$) as a dietary source and found to be between 15 and 30 mg/kg diet for common carp, *Cyprinus carpio* (Ogino and Yang, 1979) and rainbow trout, *Oncorhynchus mykiss* (Ogino and Yang, 1978), 20 mg/kg diet for channel catfish, *Ictalurus punctatus* (NRC, 2011), 20–25 mg/kg diet for red drum, *Sciaenops ocellatus* (Gatlin III et al., 1991), 37–67 mg/kg diet for Atlantic salmon, *Salmo salar* (Maage and Julshamn, 1993), and 30 mg/kg diet for Nile tilapia, *Oreochromis niloticus* (Eid and Ghonim, 1994).

The Zn absorption and bioavailability from diets are dependent on its existence and chemical form, the quality of the dietary protein source, and the presence of dietary phytate and tricalcium phosphate (Lonnerdal, 2000; Tan and Mai, 2001; Hossain et al., 2003; do Carmo e

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SÁ et al., 2005; Faiz et al., 2015; Swain et al., 2016). Besides, the inorganic Zn is easily excreted into the water resulting in deterioration of water quality (Swain et al., 2016). Hence, Zn bioavailability to fish from Zn complexes was therefore higher than that observed for $ZnSO_4$ or ZnO, especially in plant-origin diets (Ashmead, 1992). In this regard, Tan and Mai (2001) stated that inorganic Zn forms such as ZnO, $ZnSO_4$, and zinc carbonate ($ZnCO_3$) have shown a lower rate of Zn absorption in fish intestines than methionine-chelated Zn (Zn-Met).

Nanoparticles are a cluster of small atoms, 1–100 nm in size, with dissimilar properties compared to ordinary-sized materials (Anand Raj and Jayalakshmy, 2015). The application of nanotechnology has appeared in countless sectors of aquaculture and aqua-feeds (Kuzma, 2006; Handy, 2012; Abdel-Tawwab et al., 2018; Abdel-Tawwab et al., 2019). Accordingly, recent studies have been focused on using eco-friendly methods as biogenic or green methods using microorganisms, enzymes, plants or their extracts, and algae for the synthesis of nanoparticles instead of conventional methods, as it does not need intracellular synthesis and multiple phases of purification (Sinha et al., 2009; Anand Raj and Jayalakshmy, 2015; Asaikkutti et al., 2016; Gawade et al., 2017). Previous studies used ginger (*Zingiber officinale*) extract for the synthesis of silver and copper nanoparticles (Singh et al., 2011; Ipsa and Nayak, 2013; Priyaa and Kumidini, 2014) but there is no research about the application of dietary supplementation of biogenic synthesized ZnO-NP using ginger extract in fish diets.

On the other hand, the use of clay-chelated minerals in fish diets is another strategy to improve the bioavailability of minerals (Hu et al., 2008; Yildırım et al., 2009; Hassaan et al., 2020a). In particular, kaolinite, a safe feed additive, is silicate mineral clay with chemical formula $Al_2Si_2O_5(OH)_4$ (Deer et al., 1992). The majority of kaolinite consisted of kaolin, which reduced the absorption of dangerous toxins (Phillips, 1999; Dominy et al., 2004), pathogenic microorganisms, and heavy metals (Beck et al., 2015; Harikrishnana et al., 2018) when added to animal diets. Moreover, it could regulate the nutrient release, as previously recorded on clay mineral, zeolite (Zhang et al., 2011; Rahimi et al., 2012). For this reason, attention should be paid to the synthesis of ZnO supported on kaolinite (ZnO-K) to monitor the rapid release of Zn from the fish intestine and to improve the efficiency of the Zn absorption in fish intestine (Hu et al., 2014; Kutláková et al., 2015).

Due to its fast growth rate and easy adaptation to commercial diets, Nile tilapia, *Oreochromis niloticus* (L.), is one of the most popular farmed fish in many countries worldwide (El-Sayed, 2019). Despite all these favorable properties, the potential production of Nile tilapia cannot be achieved without satisfaction of its Zn requirement via using the more efficient Zn form. Therefore, to develop feeds formulation that properly meet fish Zn requirements, it is necessary to carry out research that takes into consideration not only performance but also immunity, and oxidants/antioxidants aspects. Therefore, the present study was carried out to assess the efficacy of ZnO supported on kaolinite (ZnO-K), biogenic ZnO nanoparticles (ZnO-NP), or mineral Zn ($ZnSO_4$) on growth, intestinal morphometry, digestive enzymes, hemato-biochemical and oxidative stress biomarkers, and gene expression in Nile tilapia, *O. niloticus*.

2. Materials and methods

2.1. Synthesis of biogenic of ZN-NP by using ginger extract

Fresh ginger (*Z. officinale*) roots purchased from a local market in Egypt, and its water extraction was done according to Anand Raj and Jayalakshmy (2015) with some modification. Briefly, after washing with double distilled water, it was cut into small pieces weighing 5 g and dried in hot oven at 60 °C for an hour. The cut pieces mashed well using mortar, adding 25 ml double water. The solution filtered using Whatman No.1 filter paper and the extract stored at 4 °C.

A 10 ml ginger extract was added to the solution of 0.1 M of zinc acetate dehydrate and the pH of 12 was maintained by 2 M sodium hydroxide. After that, the mixture was stirred for 2 h until a white precipitate was formed and centrifuged at 10,000 x *g* for 10 min. The supernatant was discarded and the pellet was washed with double distilled water and dried in a hot air oven at 100 °C overnight. Then, the white powder precipitation was collected carefully to be characterized by using the UV-spectrophotometer in the wavelength region 300 to 800 nm. The crystalline phase of the biosynthesized ZnO-NP was analyzed by X-Ray Diffraction (XRD) and its morphology and size were estimated by scanning electron microscope (SEM) (JEOL, JSM-5200), and transmission electron microscopy (TEM) (Jeol-Jem-2100 HRTEM, Japan). The obtained powder of the biosynthesized ZnO-NP using ginger extract herein is hexagonal and agglomerated with a particle size range of 13.26–47.69 nm.

2.2. Synthesis of ZnO supported on kaolinite

The synthesis of ZnO supported on kaolinite (ZnO-K) was made by using a hydrothermal method according to Hrenovic et al. (2012) with some modification. Briefly, adding 0.1 M zinc acetate dehydrate to 10 g of kaolinite under stirring at room temperature. After 12 h of stirring, aqueous solution of NaOH (5 M) was slowly added drop-wise to the solution under stirring until the pH of the solution reached to 13. The precipitate was oven-dried at 95 °C for 120 min, centrifuged, washed twice with double-distilled water, and dried at 50 °C.

2.3. Diets preparation and fish culture

Three isonitrogenous and isoenergetic experimental diets were formulated to meet the requirements of Nile tilapia (NRC, 2011). A 30 mg Zn/kg diet was supplemented to each diet with different forms i.e. biogenic ZnO-K, ZnO-NP, or $ZnSO_4$, respectively (Table 1) based on the Zn requirement for Nile tilapia (Eid and Ghonim, 1994). Experimental diets were prepared using pelleting hand-noodle maker (2-mm), and then kept in cellophane bags at −4 °C until use. The Zn concentrations in experimental diets; ZnO-K, ZnO-NP, and $ZnSO_4$ after preparation were 157.49, 157.41, and 158.58 mg/kg diet, respectively. The proximate chemical analysis of the experimental diets was determined according to AOAC (2005).

Nile tilapia, *O. niloticus,* were acclimatized in fiberglass tanks for 15 days after obtained from the fish farm of National Institute of Oceanography and Fisheries, during which fish were fed a commercial diet (30% crude protein) up to apparent satiation four times a day. After acclimation, fish (2.12 ± 0.02 g) were stocked in twelve 250-L fiberglass tanks at a density of 25 fish per tank in triplicates. All tanks were supplied with continuous aeration through air-stones, connected to a central air compressor. All fish were hand fed on the tested diets up to apparent satiation four times per day at 9:00, 12.00, 14.00, and 16.00 h for 12 weeks.

Water quality was monitored every day throughout the feeding trial at 15 cm depth from each tank to evaluate the water quality parameters. Dissolved oxygen (DO) and temperature (°C) were measured in-site using a portable oxygen meter (Jenway, London, UK). The pH was measured using a pH meter (Digital Mini-pH Meter, USA). Unionized ammonia (NH_3) was measured using special kits (HACH Co., Loveland, USA). The ranges of the above-mentioned parameters were 26.5–29.7 °C, 6.2–6.5 mg/L, 7.6–7.8, and 0.135–0.242 mg/L for water temperature, DO, pH, and NH_3, respectively. These values are kept-up within the acceptable ranges for fish farming according to Boyd and Tucker (2012).

After the feeding trial, fish from each tank were collected, counted, and final body weight of individual fish was recorded. Parameters of growth performance and feed utilization were calculated according to Abdel-Tawwab et al. (2020a, 2020b) as follows:

Weight gain (g) = W2–W1;

Specific growth rate (SGR; %g/day) = 100 (Ln W2 – Ln W1)/T;

Table 1
Ingredients and proximate chemical composition (g/kg diet dry matter) of experimental diets containing different Zn forms.

Ingredients	
Fish meal (60% CP)	130
Soybean meal (44% CP)	350
Corn gluten (62% CP)	80
Yellow corn (8.5% CP)	280
Rice polishing (13% CP)	99.7
Fish oil	40
Zinc-free premix[1]	20
Zinc form	0.3
Total	1000
Chemical analysis	
Moisture	8.82
Crude protein	316.1
Total lipid	72.65
Ash	59.92
Crude fiber	51.30
NFE[2]	399.97
Gross energy[3]	18.86

[1] Vitamin and mineral mixture kg^{-1} of mixture contains: 4800 I.U. Vit A, 2400 IU cholecalciferol (vit. D), 40 g Vit E, 8 g Vit K, 4.0 g Vit B_{12}, 4.0 g Vit B2, 6 g Vit B6, 4.0 g, Pantothenic acid, 8.0 g Nicotinic acid, 400 mg Folic acid, 20 mg Biotin, 200 g Choline, 4 g Copper, 0.4 g Iodine, 12 g Iron, 22 g Manganese, 22 g Selenium; folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39% Na, 61% Cl), 3.077 g; ferrous sulfate ($FeSO_4.7H_2O$, 20% Fe), 65 mg.

[2] NFE (Nitrogen free extract) =100 - (crude protein % + lipid % + ash % + fiber %).

[3] Gross energy calculated using gross calorific values of 23.63, 39.52 and 17.15 kj/g for protein, fat, and carbohydrate, respectively according to Brett (1973).

Where W1 and W2 are the initial and final weights, respectively, and T is the experimental period (days);
Feed intake (g feed/fish) = the summation of feed consumed by fish throughout the experiment/fish number per aquarium;
Feed conversion ratio (FCR) = feed intake/fish weight gain;

Fish survival (%) = 100 (final fish number/initial fish number).

2.4. Intestinal histomorphometry

At the end of feeding trial, three fish from each tank were anesthetized, dissected, and intestine samples were randomly taken. Afterwards, samples were washed in phosphate buffer saline (PBS), fixed in 10% formalin for 24 h, dehydrated in ascending grades of alcohol, and cleared in xylene. Then, samples were embedded in paraffin wax (congealing point 58–60 °C). The longitudinal and transverse sections each of 6 μm thickness were cut by using a Rotatory Microtome (Reichert Technologies, NY, USA) and stained in hematoxylin and eosin (H&E) according to the standard procedure (Bancroft and Stevens, 2010). Additionally, some of these sections were stained with Alcian blue pH 2.5 to elucidate the area percentage of Alcian blue positive secreting goblet cells (Sheehan and Hrapchak, 1980). The tissue sections were examined under light microscope equipped with full HD microscopic camera and image analysis software (Leica Microsystem, Germany). The mean villus height (measured from the base to the top) measured by image analysis software for statistical analysis. Absorption surface area (ASA) was calculated as follows:

ASA (mm2) = villus height x villus width

2.5. Blood and tissues sampling

Fish were fasted for a period of 24 h immediately before the sampling and anesthetized by buffered tricaine methane sulphonate (MS-222; 25 mg/L, Sigma Aldrich, Egypt). The blood was collected from the caudal veins (3 fish per tank) using heparinized tubes (1600 UI/mL) to determine hematological parameters. The second blood sample was collected from the caudal vein using non-heparinized 3-ml syringes and was allowed to clot for 30 min at 4 °C then centrifuge at 4000 ×*g* for 15 min to obtain serum for estimating biochemical analysis.

Afterward, 3 fish from each tank were dissected and livers and intestines were taken and kept in an ice-cold plate, washed with physiological cold saline, and dehydrated by filter paper. Liver tissue was homogenized in phosphate buffer saline (PBS) as described by Abdel-Latif et al. (2020). The liver of fish in each treatment were weighed and grinded in glass homogenizer tubes with ice-cold saline (0.1 g of liver was added to 0.9 mL saline, pH 7.0), then centrifuged at 3000 x *g* for 20 min at 4 °C to obtained supernatant, which was kept at −80 °C till be used in the evaluation of the oxidative status. At the same time, a piece of liver (3 fish/tank) was collected and stored in 2 mL tubes then were thrown in liquid nitrogen and finally frozen at −80 °C. Samples of the middle intestine (3 fish/tank) were immediately homogenized in 10 volumes of ice-cold physiological saline solution and centrifuged at 5000 *g* for 15 min at 4 °C; then, the supernatant was stored for measuring the activity of digestive enzymes.

2.6. The measurement of digestive enzymes activity and cholecystokinin content

The activities of chymotrypsin and trypsin were estimated according to Hummel (1959). Activities of lipase and amylase were determined as described by Zamani et al. (2009) and Bernfeld (1951), respectively. According to Maton et al. (1984) method, the cholecystokinin content (CCK) in fish intestine was determined.

2.7. Hemato-biochemical analysis

Hematocrit (Hct), hemoglobin (Hb), erythrocyte counts (RBCs), and total count of white blood cells (WBCs) were estimated according to Rawling et al. (2009). Serum total protein (TP) and albumin (ALB) were determined according to Doumas et al. (1981) and serum globulin (GLO) was calculated by subtracting the serum albumin from the total serum protein. The determination of alkaline phosphatase (ALP) activity was estimated according to Tietz et al. (1983), while activities of aspartate (AST), and alanine aminotransferase (ALT) were determined by Reitman and Frankel (1957).

2.8. Determination of hepatic oxidative stress biomarkers

Activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) as well as total antioxidant capacity (T-AOC) and malonaldehyde (MDA) levels were measured using diagnostic kits (Bio-diagnostics, Giza, Egypt) following the manufacturer's instructions according to the methods of Nishikimi et al. (1972), Aebi (1984), Moin (1986), Benzie and Strain (1996), and Uchiyama and Mihara (1978), respectively.

2.9. Gene expression

After fish anesthetized as described before, liver samples were removed and homogenized by tissue homogenizer (QIAGEN GmbH, QIAGEN Strasse 1, Hilden, Nordrhein-Westfalen-40,724, Germany). Total ribonucleic acid (RNA) was extracted from the tissues, using RNeasy® Mini kit (QIAGEN, Cat No. 74104), based on the manufacturer's protocol. The cDNA was synthesized from 1000 ng of the total RNA using the protocol of high capacity cDNA Reverse Transcription

Table 2
Oligonucleotide name and sequence of qRT-PCR primers used in this experiment.

Gene	Primers	GenBank no.
18 s rRNA[ǂ]	Forward 5- > 3: GGTTGCAAAGCTGAAACTTAAAGG	AF497908.1
	Reverse 5- > 3: TTCCCGTGTTGAGTCAAATTAAGC	
SOD[†]	Forward 5- > 3: CATGCCTTCGGAGACAACAC	AY491056.1
	Reverse 5- > 3: ACCTTCTCGTGGATCACCAT	
CAT[‡]	Forward 5- > 3: AGCTCTTCATCCAGAAACGC	JF801726.1
	Reverse 5- > 3: GACGTCAGGCGTCACATCTT	
IgM-2[*]	Forward 5- > 3: CCACTTCAACTGCACCCACT	KC677037.1
	Reverse 5- > 3: TGGTCCACGAGAAAGTCACC	

18 s rRNA[ǂ] = 18 s ribosomal RNA.
SOD[†] = superoxide dismutase.
CAT[‡] = catalase.
IgM-2[*] = immunoglobin M-2.

Kit (Applied Biosystems, MA, USA, Cat# no.4368813), then cDNA was stored at −80 °C for further molecular analyses.

The primers used for amplifications of the gene, which encodes Ig M-2, SOD, CAT and 18S ribosomal RNA (18S rRNA; as reference gene) were used for quantifying the expression of the target genes using real time PCR (qRT-PCR) methodology (Table 2). Quantitative PCR reaction contained 2.5 μg/μl cDNA, 12.5 μl SYBR Green PCR Master Mix (QuantiTect SYBR Green PCR Kit, QIAGEN), 0.3 μM of each of forward and reverse primers and a final volume of 25 μl was made by sterile double distilled water. The reaction was run on an Applied Biosystem 7500 Real time PCR Detection system (Applied Biosystems) under the conditions of 95 °C for 10 min followed by 45 cycles of 95 °C for 20 s, 60 °C for 20 s and 72 °C for 20 s. The quantitative real time of the examined genes were standardized using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) and the real-time PCR program was analyzed and estimated according to the methods described by Yuan et al. (2006).

2.10. Analysis of Zn concentrations in diets and fish muscles

Samples of diets and fish muscles were randomly taken and dried in a drying oven at 105 °C for 48 h. After that, samples were homogenized and digested in concentrated H_2SO_4 as described in Abdel-Tawwab (2016). Zinc concentrations in fish muscles were determined using atomic emission spectrophotometer (IRIS Advantage, Thermo Jarrel Ash Corporation, Boston, USA) using standard Zn concentrations.

2.11. Data analysis

Data were tested for homogeneity and normality tests. Afterwards, data were analyzed using one-way analysis of variance and the differences among means were made by using Duncan's multiple range test using SAS ANOVA procedure (SAS, version 6.03, Soft Inc., Tusla, OK, USA, SAS, 1993). The differences at $P < 0.05$ were considered significant. The values are presented as means ± standard error of the mean (SEM).

3. Results

3.1. Growth performance and feed utilization

Growth performance was significantly ($P < 0.05$) higher in fish fed dietary ZnO-K and ZnO-NP than inorganic form ($ZnSO_4$). The highest growth performance was observed in fish ZnO-K in diets, which consumed more feed (32.3 g feed/fish) as compared with other Zn forms. No significant difference in FCR values was observed among treatments and its ranged was 1.32–1.38 (Table 3). Also, no significant ($P > 0.05$) difference was observed in fish survival among different treatments and its range was 96.7–100% suggesting that there was no Zn toxicity to affect fish health (Table 3).

Table 3
Growth performance and feed utilization of Nile tilapia, *O. niloticus,* fed diets containing different Zn forms for 12 weeks.

	Experimental diets			SEM
	ZnO-NP	ZnO-K	$ZnSO_4$	
Initial body weight (g)	2.3	2.1	2.2	0.020
Final body weight (g)	23.9[b]	26.6[a]	21.0[c]	0.497
Weight gain (g)	21.6[b]	24.5[a]	18.8[c]	0.490
Specific growth rate (%/day)	2.79[b]	3.02[a]	2.69[c]	0.026
Feed intake (g feed/fish)	28.9[b]	32.3[a]	25.9[c]	0.420
Feed conversion ratio	1.34	1.32	1.38	0.049
Fish survival (%)	96.7	98.3	100	0.66

Means followed by different letters in the same row are significantly different ($P < 0.05$).

3.2. Intestinal histomorphology parameters

The examination of stained sections of fish intestine showed that the mucosa was modified into longitudinal finger-like projections, villi, which extended into the intestinal lumen and lined with simple columnar epithelial enterocytes. It is also observed that goblet cells were inserted among the epithelial cells (Fig. 1 A, B, C). The villus height/width was significantly ($P < 0.05$) higher in fish fed either ZnO-K or ZnO-NP than $ZnSO_4$ form. Highest values of villus length, width, and absorption area were obtained in fish fed ZnO-K followed by those fed a ZnO-NP containing diet (Table 4; Fig. 1 A, B, C). A significant highest percentage of Alcian blue-positive goblet cells ($P < 0.05$) was observed in fish fed ZnO-K, meanwhile lowest one was obtained in fish fed $ZnSO_4$ form (Table 4; Fig. 2).

3.3. Digestive enzymes activity and cholecystokinin content

The activities of intestinal digestive enzymes were significantly ($P < 0.05$) affected by dietary Zn form, where highest values of chymotrypsin, trypsin, lipase, and amylase were observed in fish fed ZnO-K followed by those fed ZnO-NP but their lowest values were observed in fish fed $ZnSO_4$ form (Table 5). On the other hand, the highest cholecystokinin content was also recorded in fish fed ZnO-K; meanwhile its lowest content was observed in fish fed $ZnOS_4$ (Fig. 3).

3.4. Immune-biochemical parameters

No significant differences ($P > 0.05$) were found in values of RBCs, Hb, and Hct among experimental diets; meanwhile WBCs count was significantly ($P < 0.05$) affected by dietary Zn form and its highest count was obtained by fish fed ZnO-K containing diet (Table 6). It is also noted that serum TP, ALB, and GLO levels were higher in ZnO-K or ZnO-NP treatments as compared with $ZnSO_4$ form ($P < 0.05$; Table 6). Additionally, serum ALP activity was significantly ($P < 0.05$) higher in fish fed ZnO-K followed by those fed ZNO-NP, while lowest activity was observed in fish fed mineral $ZnSO_4$ form. On the other hand, highest activities of serum ALT and AST were observed in fish fed $ZnSO_4$ containing diet as compared with other Zn forms ($P < 0.05$; Table 6). The transcripts expression of IgM-2 gene was significantly ($P < 0.05$) up-regulated in the liver of fish fed ZnO-K in diets, while its lowest gene expression was observed in fish fed mineral $ZnSO_4$ form (Fig. 4).

3.5. Hepatic oxidative stress biomarkers

Compared to the $ZnSO_4$ form, significant higher activities of CAT, SOD, and GPx as well as T-AOC value accompanied with lower value of MDA were observed in fish fed a diet containing ZnO-K followed by a ZnO-NP containing diet ($P < 0.05$; Table 7). On the other hand, the

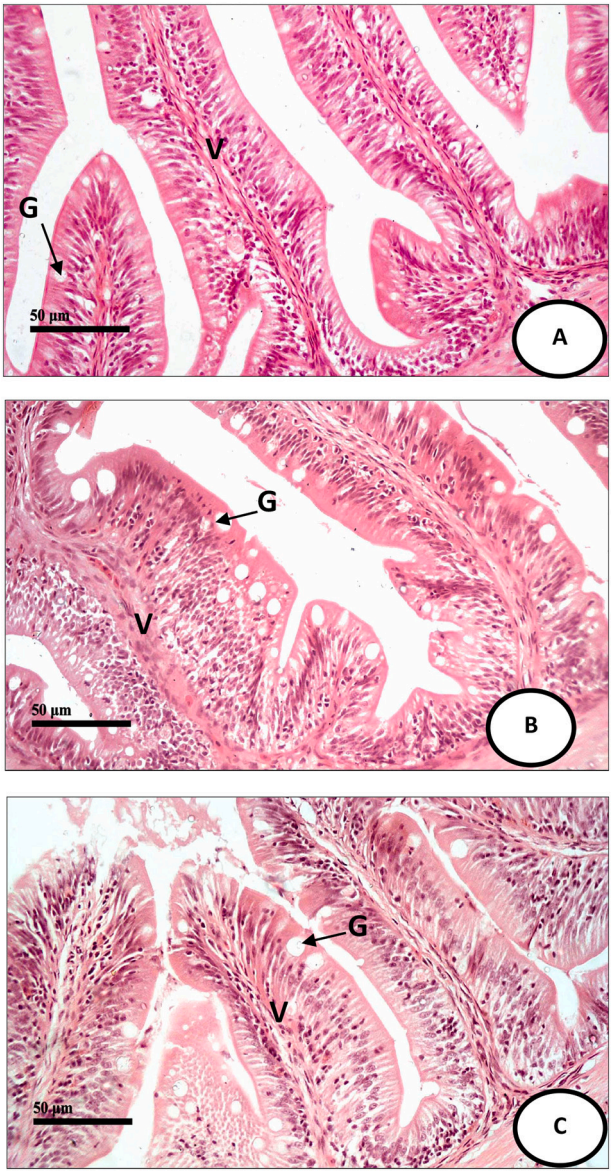

Fig. 1. Photomicrographs of small intestine sections of Nile tilapia, *O. niloticus,* stained with Hematoxylin & Eosin (H&E) showing the branching anastomosing mucosal villi (V) with goblet cells (G). (A) ZnO-NP, (B) ZnO-K, and (C) $ZnSO_4$ (H & E. x400); scale bar = 50 μm.

Table 4
Intestinal morphology of Nile tilapia, *O. niloticus,* fed diets containing different Zn forms for 12 weeks.

	Experimental diets			SEM
	ZnO-NP	ZnO-K	$ZnSO_4$	
Villus width (μm)	302.3^{b}	312.2^{a}	284.2^{c}	14.72
Villus height (μm)	900.9^{b}	942.5^{a}	765.5^{c}	11.12
Absorption surface area (ASA; mm^2)	0.272^{b}	0.294^{a}	0218^{b}	1.12
Goblet cells area (%)	7.58^{b}	8.88^{a}	6.80^{c}	0.27

Means followed by different letters in the same row are significantly different ($P < 0.05$).

transcripts expression of SOD and CAT genes were significantly ($P < 0.05$) up-regulated in the liver of fish fed a diet containing ZnO-K, whereas its lowest expression was observed in fish fed mineral $ZnSO_4$ form (Fig. 5).

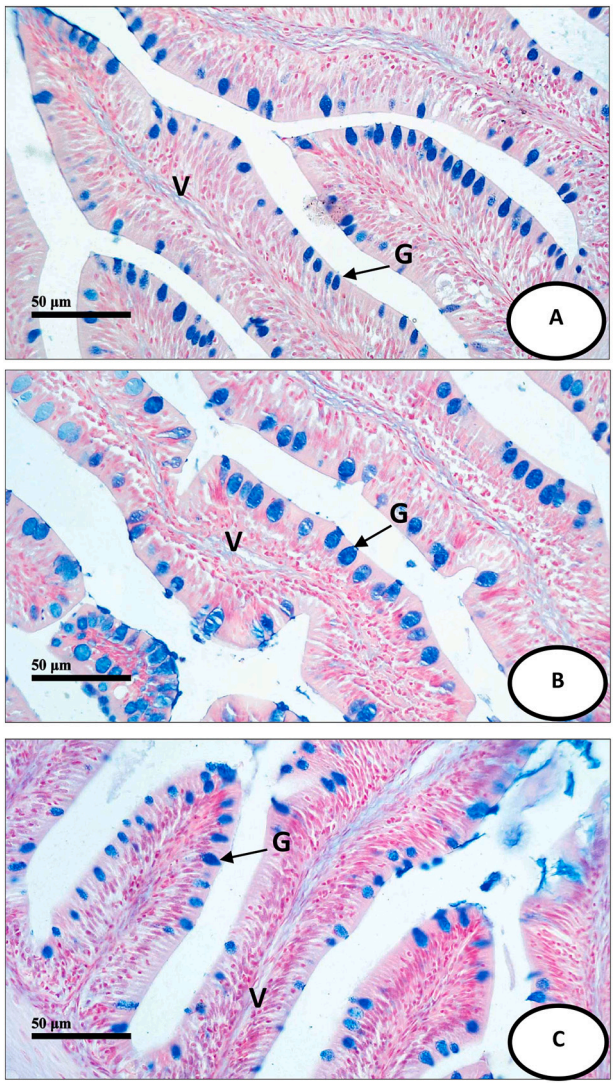

Fig. 2. Photomicrographs of small intestine sections of Nile tilapia, *O. niloticus,* stained with Alcian Blue (pH 2.5) stain showing the branching anastomosing mucosal villi (V) with numerous Alcian Blue positive goblet cells (G). (A) ZnO-NP, (B) ZnO-K (C), and $ZnSO_4$ (Alcian Blue, x 400); scale bar = 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 5
Activities of intestinal digestive enzymes (U/g tissue) of Nile tilapia, *O. niloticus,* fed diets containing different Zn forms for 12 weeks.

	Experimental diets			SEM
	ZnO-NP	ZnO-K	$ZnSO_4$	
Chymotrypsin	6.19^{b}	7.85^{a}	5.49^{c}	0.28
Trypsin	34.85^{b}	41.10^{a}	28.75^{c}	1.20
Lipase	1116.2^{b}	1190.2^{a}	909.5^{c}	248.73
Amylase	722.0^{b}	908.5^{a}	673.0^{c}	14.53

Means followed by different letters in the same row are significantly different ($P < 0.05$).

3.6. Zinc residues in fish muscles

Zinc concentrations in fish muscles are shown in Fig. 6. The highest Zn value was observed in muscles of fish fed ZnO-K containing diets followed by that of ZnO-NP form. The lowest Zn residue was observed in muscles of fish fed mineral Zn form ($ZnSO_4$).

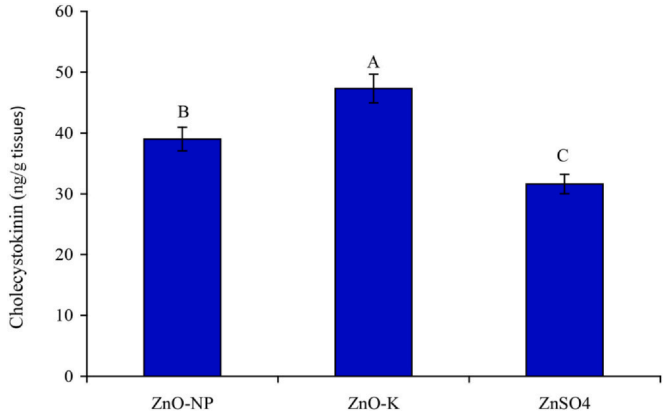

Fig. 3. Cholecystokinin (ng/g tissues) of Nile tilapia, *O. niloticus,* fed diets containing different Zn forms for 12 weeks. Bars having different letters are significantly different at $P < 0.05$.

Table 6
Hemato-biochemical parameters of Nile tilapia, *O. niloticus,* fed diets containing different Zn forms for 12 weeks.

	Experimental diets			SEM
	ZnO-NP	ZnO-K	$ZnSO_4$	
WBCs ($\times 10^3$ cmm^{-1})	68.20^a	68.94^a	61.10^b	0.947
RBCs ($\times 10^6$ cmm^{-1})	2.72	2.79	2.42	0.058
Hemoglobin (g dL^{-1})	9.63	9.60	9.00	0.196
Hematocrit (%)	21.93	24.23	22.13	1.032
Total protein (g L^{-1})	4.42^a	4.51^a	3.40^b	0.078
Albumin (g L^{-1})	1.87^a	1.94^a	1.51^b	0.042
Globulin (g L^{-1})	2.55^a	2.57^a	1.89^b	0.073
ALP (UL^{-1})	32.9^b	43.8^a	27.8^c	1.972
ALT (UL^{-1})	41.04^b	39.18^b	46.63^a	1.585
AST (UL^{-1})	11.17^b	10.31^b	13.37^a	0.610

Means followed by different letters in the same row are significantly different ($P < 0.05$).

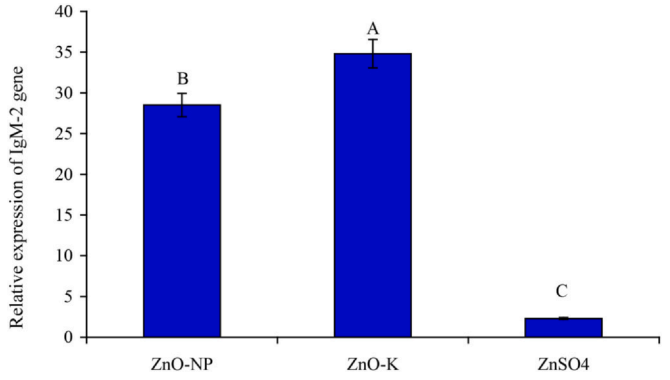

Fig. 4. Relative expression of immunoglobulin-2 (IgM-2) gene/18 s rRNA of Nile tilapia, *O. niloticus,* after feeding diets with different Zn forms for 12 weeks. Bars having different letters are significantly different at $P < 0.05$.

4. Discussion

4.1. Fish growth and feed utilization

Zinc occurs naturally in different organic, inorganic, and mineral forms (Huang et al., 2015; Abdel-Tawwab, 2016; Pagano et al., 2017; Capillo et al., 2018). These different Zn forms have different properties and functions that affect its availability to fish (Wang and Wang, 2015; Shahpar and Johari, 2019). Therefore, it is interesting to evaluate the effects of different Zn forms in aqua-feeds on fish performance. In the present study, fish fed a ZnO-K containing diet achieved highest growth and feed utilization as compared with other dietary Zn forms. These results may be because kaolinite acts as an effective carrier between the interlayer of kaolinite, which regulates the release of ZnO in intestinal tract for enhancing the intestinal function, digestibility, and growth performance of fish (Aguzzi et al., 2007; Zhang et al., 2011; Rahimi et al., 2012; Hu et al., 2014). Moreover, kaolinite has the ability to adsorb anti-nutritional factors contained in plants-protein sources as soybean in diets used in the current experiment (Table 1). In similar studies, ZnO supported on zeolite or montmorillonite (ZnO-MMT) improved the intestinal function and the growth performance of Nile tilapia (Hu et al., 2014), broilers (Hu et al., 2013), and weaned pigs (Hu et al., 2012). Buentello et al. (2009) reported that hybrid striped bass utilized Zn from zinc proteinate form around 1.7 times more than that of mineral $ZnSO_4$ form. Tan and Mai (2001) found that Zn bioavailability from Zn-methionine form was three times more than that of mineral $ZnSO_4$ form for *Haliotis discushannai*. In contrast, Dekani et al. (2019) compared the effects of dietary organic Zn (Zn-proteinate, Bioplex Zn®), nanoparticulate zinc (ZnO-NPs), or mineral zinc ($ZnSO_4$) on growth performance, whole-body proximate composition, and antioxidant enzymes of common carp. They found no significant difference in weight gain between the control group and fish received different Zn forms and levels, but highest SGR value was observed in fish received 100 mg/kg diet of $ZnSO_4$. Shahpar and Johari (2019) investigated the effects of organic zinc (Zn-proteinate, Bioplex Zn®), ZnO-NP, or $ZnSO_4$ on performance, survival, and body composition of larval rainbow trout. They found no significant difference in fish growth.

The growth improvement in fish fed ZnO-NP in diets as compared with the control diet ($ZnSO_4$) may be regarded to the higher intestinal absorption, bioavailability and catalytic activities (Albrecht et al., 2006; Dube et al., 2010; Alishahi et al., 2011; Zhou et al., 2006; Swain et al., 2016; Onuegbu et al., 2018). Additionally, ZnO-NP stimulated the somatic growth through stimulation the synthesis of DNA, RNA, growth hormone, and cell division in fish body (Siklar et al., 2003). Likewise, ZnO-NP enhanced the growth performance of grass carp, *Ctenopharyngodon idella*, common carp, Nile tilapia, and rohu, *Labeo rohita* (Faiz et al., 2015; Tawfik et al., 2017; Chupani et al., 2017; Swain et al., 2019). Therefore, ZnO-K could be considered as the optimum form of Zn for encouraging the fish performance because of the continuous and slow release of Zn leading to high Zn availability as compared with other Zn forms.

Table 7
Hepatic antioxidant activities of Nile tilapia, *O. niloticus,* fed diets containing different Zn forms for 84 days.

	Experimental diets			SEM
	ZnO-NP	ZnO-K	$ZnSO_4$	
CAT (U/g protein)	151.8^b	199.7^a	149.4^c	2.12
SOD (U/g protein)	108.25^b	147.4^a	78.3^c	1.51
T-AOC (U/g protein)	17.1^b	22.9^a	15.05^c	1.23
GPx (U/g protein)	693^b	920.2^a	610.15^c	5.02
MDA (nmol/g tissue)	2.01^b	1.60^c	2.95^a	0.02

Means followed by different letters in the same row are significantly different ($P < 0.05$).

4.2. Intestine histomorphology

Villus width/length and absorption area are essential indicators of intestinal morphology due to their vital role in nutrient absorption (Wang et al., 2017). Also, intestinal mucin is synthesized by exocytosis of the goblet cells, has high molecular weight glycoprotein and covered the intestinal mucosa for their surface protection against any toxins (Strugala et al., 2003; Pelaseyed et al., 2014). In our results, the addition of ZnO-K or ZnO-NP to fish diets increased the villus width/length

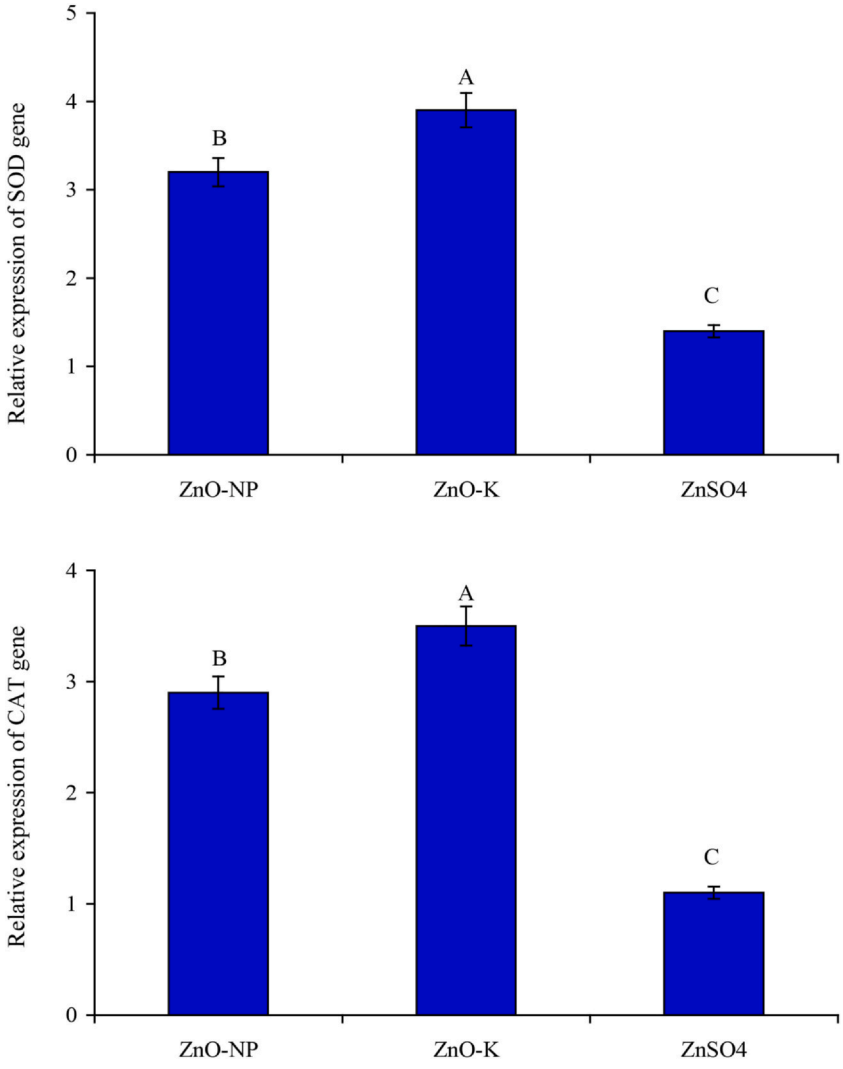

Fig. 5. Relative expression of superoxide dismutase (SOD) and catalase (CAT) genes/18 s rRNA of Nile tilapia, *O. niloticus,* after feeding diets containing different Zn forms for 12 weeks. Bars having different letters are significantly different at $P < 0.05$.

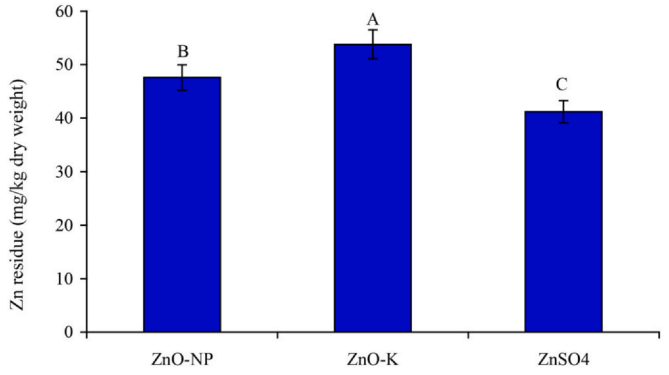

Fig. 6. Zinc residue (mg/kg dry weight) in muscles of Nile tilapia, *O. niloticus,* after feeding diets with different Zn forms for 12 weeks. Bars having different letters are significantly different at $P < 0.05$.

and the percentage of acidic mucin producing goblet cell in the fish intestine, and their highest values were found in fish fed a ZnO-K containing diet. This improvement may be due to the presence of effective carrier kaolinite that could continual slow the release of Zn into the intestinal tract causing better nutrients absorption (Hu et al., 2014). Besides, diets containing kaolinite could maintain the epithelial integrity and improved the intestinal morphology function by reducing the turnover of villus cells and increasing mucin secretion as shown previously in broiler chicken fed a kaolin-containing diet (de Lemos et al., 2015). Furthermore, the inclusion of ZnO-NP herein exhibited such intestinal changes as the increase of villus width/length and acidic mucin producing goblet cell percentage. That may be regarded to the small average size of ZnO-NP, with a large surface area facilitating the intestinal nutrient absorption and digestibility. In a similar study, Hu et al. (2014) found an increase in the intestinal villus length of Nile tilapia fed a diet with ZnO supported on zeolite (ZnO-Z).

4.3. Intestinal digestive enzymes

Activity of digestive enzymes can be considered a biochemical indicator of feeding activity in fish (Silva et al., 2019). Secretion of these enzymes especially proteases, as well as feed intake, is regulated by neural and hormonal factors (Ji et al., 2015; Konturek et al., 2003). One of these factors is cholecystokinin (CCK), which is secreted in the intestine in response to feed intake and acts on gallbladder control, secretion of pancreatic enzymes, reduction in gastric emptying and satiety (Einarsson et al., 1997; Micale et al., 2014; Santos et al., 2020a, 2020b). In the present study, activities of chymotrypsin, trypsin, lipase, and amylase as well as CCK content were significantly higher in fish fed ZnO-K followed by ZnO-NP enriched diets. These results may be

associated with the adhering ability of kaolinite in ZnO-K to the mucous for defending, strengthen the intestinal mucosa and helping in the regeneration of the intestinal epithelium (Albengres et al., 1985; Girardeau, 1987; Hu et al., 2008). In addition, the secretion of digestive enzymes could be altered by kaolinite and ZnO ability in adapting pH and the ionic composition of gastrointestinal fluids (Martin-Kleiner et al., 2001; Hassaan et al., 2020a). Corresponding to our findings, adding Cu^{2+}- exchanged montmorillonite or ZnO-Z in Nile tilapia diets elevated the secretion of amylase, lipase, and alkaline phosphatase (Hu et al., 2008, 2014). In a similar study, Asaikkutti et al. (2016) indicated that biogenic synthesis of Mn_3O_4 nanoparticles using *Ananas comosus* elevated the secretion of protease, amylase, and lipase of *Macrobrachium rosenbergii*.

4.4. Immune-biochemical variables

Biochemical parameters are considered as essential indicators for fish health, immunity, and the physiological stress response (Faggio et al., 2014; Dawood et al., 2015; Abdel-Tawwab, 2016; Hassaan and Mohammady, 2019; Hassaan et al., 2020b). The present study showed no significant differences in values of RBCs, Hb, and Hct in fish fed different dietary Zn forms, whereas highest WBCs count was observed in fish fed a diet supplemented with ZnO-K followed by ZnO-NP. The WBCs count is considered as the first line of defense, which could be related to the stronger innate resistance and adaptive immunity (Divyagnaneswari and Christybapita, 2007).

It is clear in the present study that fish fed either ZnO-K or ZnO-NP containing diets improved TP, ALB, and GLO values as compared with $ZnSO_4$ containing diet. These results may be probably due to the stimulation of DNA, formation of ribosomes, and the proliferation of protein synthesis in the liver tissues (Sakr et al., 2005; Akrami et al., 2015). Gopal et al. (1997) found that serum GLO in common carp increased by the supplementation with ZnO-NP. Abdel-Khalek et al. (2015) reported that ZnO-NP could potentially interact with cell macromolecules as protein, lipid, and DNA affecting protein metabolism and/or alert gene expression for many vital enzymes higher than $ZnSO_4$.

The lysozyme activity is exceptionally a widespread as a humoral component associate in the innate immune system that is important for protecting against fish disease (Kaya et al., 2016). In fish, in terms of structural and physiological features, IgM is considered as an effective immune molecule (Akrami et al., 2015). The IgM produced by the plasma cells of the spleen and lymph nodes and secreted into serum. In the present study, the transcripts expression of IgM-2 gene was significantly ($P < 0.05$) up-regulated in the liver of fish fed a diet containing ZnO-K compared with other Zn forms, while lowest gene expression was observed in fish fed mineral $ZnSO_4$ form. This up-regulation may be due to the continuous and slow release of Zn from ZnO-K form as compared with other Zn forms. In a similar study, Gharaei et al. (2020) found that lysozyme levels significantly increased in beluga (*Huso huso*) fed chitosan–ZnO-NP supplemented diets. These results indicated that Zn have positive effects on immune system including the natural development and function of the mediate cells of nonspecific receptors such as neutrophils. The same results obtained by Awad et al. (2019), who noted that gene expression of IgM was upregulated for Nile tilapia fed diet 30 mg ZnO-NP per kg diet as well as the improvement in serum IgM.

ALPs are a group of zinc-dependent enzymes and present in most tissues of the body whose induce transfer activity, catalytic activity, and generally leakage from the liver (Gharaei et al., 2011; Estaki et al., 2014). Zn and magnesium are two important cofactors of this enzyme (Ray et al., 2017). The present study found that ALP activity was highest in fish fed ZnO-K followed by ZnO-NP enriched diets over the inorganic form ($ZnSO_4$). It is known that the high Zn availability lead to high ALP activity (Gharaei et al., 2020). However, Zn supplementation improved ALP activity of juvenile grass carp (Liang et al., 2012), hybrid tilapia, *Oreochromis niloticus* × *O. aureus* (Li and Huang, 2016), and common carp (Dekani et al., 2019).

Activities of AST and ALT are essential enzymes in cellular nitrogen metabolism, oxidation of amino acids, and liver gluconeogenesis and they could be used as a tool for detecting any toxic effect that causing liver injury or liver dysfunction (Murray et al., 2003; Abdel-Tawwab, 2016; Huang et al., 2015; Moazenzadeh et al., 2018). The higher AST and ALT activities were observed in fish fed a $ZnSO_4$ containing diet; meanwhile their lower activities were observed in fish fed ZnO-K and ZnO-NP diets. These results suggest that both Zn forms (ZnO-K and ZnO-NP) did not induce liver impairments or may be associated with production of cytokine, which protected liver cells (Mirghaed et al., 2017, 2018). Similar results were observed in common carp due to the addition of ZnO-NP in diets (Lee et al., 2014). Awad et al. (2019) observed significant rises in AST and ALT activities in Nile tilapia fed diet supplemented with 30 mg ZnO-NP per kg diet, suggested that this form of Zn have protective effects for liver cells.

4.5. Oxidative stress biomarkers

The Zn restrains the generation of reactive oxygen species (ROS) such as superoxide anion radical, hydroxyl radical, and hydrogen peroxide (Ogawa et al., 2011). The antioxidant effect of Zn may be mediated through the direct action of Zn ion, its structural role in antioxidant proteins, and modulation metallothionein induction. Direct antioxidant activity of Zn ions is associated with its binding to thiol groups and thus protects them from oxidation (Olechnowicz et al., 2018). SOD as an antioxidant enzyme can accelerate the conversion of O_2^- to $H_2O_2^-$, which is reduced to water by CAT and GPx (Ruas et al., 2008; Birben et al., 2012). However, CAT is an omnipresent tetrameric heme-containing antioxidant enzyme that accelerates the conversion of 2 molecules of H_2O_2 into H_2O and O_2 (Sharma et al., 2012). GPx catalyzes the transformation of H_2O_2 to H_2O or organic peroxides into their analogous stable alcohols by oxidation the reduced glutathione to glutathione disulfide (Manduzio et al., 2004).

Activities of CAT, SOD, and GPx as well as T-AOC value in the current study were significantly higher accompanied with low MDA level in response to dietary ZnO-K followed by ZnO-NP. These results could be linked with the up-regulation of the expression of CAT and SOD genes in fish fed ZnO-K followed by ZnO-NP diet over that fed mineral $ZnSO_4$ diet. These results may be due to the higher Zn availability from ZnO-K diet than other Zn forms. Also, this finding gives an indicator for reducing oxidative damage by free radical, which occurred during the processes of lipid peroxidation (Halliwell and Gutteridge, 2015). Wu et al. (2011) reported that the mRNA level of CAT and mu glutathione S-transferase (GST mu) in abalone increased and attained the peak at the 33.8 mg Zn/kg diet. Furthermore, Sheikh Asadi et al. (2018) reported that the increase in SOD and GPx activities enhances the activity of NADPH oxidase, which is responsible for scavenging of superoxide anion. In a similar study, Dekani et al. (2019) demonstrated that 500 mg/kg diet of ZnO-NPs significantly increased the SOD activity when compared to the respective amount of other Zn forms, while the minimum CAT activity was observed in the fish fed diets containing 500 mg/kg as $ZnSO_4$. Asaikkutti et al. (2016) found that a supplemental diet with biogenic Mn_2O_3 nanoparticles by using *A. comosus* peel extract increased the antioxidant defense system and metabolic activities of shrimp including SOD and CAT activities.

MDA is an important non-enzymatic antioxidant that has been used as a biomarker for lipid peroxidation and health condition of cell membranes (Khosravi-Katuli et al., 2018). Besides, ROS may destruct polyunsaturated lipids by producing MDA (Jafarinejad et al., 2018). The lowest level of MDA in the present study was observed in fish fed ZnO-K followed by ZnO-NP diets as compared with mineral $ZnSO_4$ form. On the other hand, Gharaei et al. (2020) found that MDA level showed no significant differences ($P > 0.05$) in fish that were fed with ZnO or chitosan–ZnO NP–supplemented diets in compared with the

control.

4.6. Zn residue in fish muscles

The present study showed that higher Zn concentration in fish muscles was observed in fish fed ZnO-K followed by ZnO-NP diets, while lowest Zn concentration was observed in fish fed mineral $ZnSO_4$ form. These results may be due to the higher Zn availability in ZnO-K than other Zn forms. On the other hand, the very small size and large surface area of ZnO-NP increased its capability to penetrate cell and nuclear membranes than ordinary $ZnSO_4$. In contrast to our finding, Hu et al. (2014) found that the addition of ZnO-Z, mix of zeolite and ZnO, or $ZnSO_4$ have no ($P > 0.05$) effects on Zn concentrations in the whole body of Nile tilapia. Shahpar and Johari (2019) found that fish fed mineral $ZnSO_4$ showed the highest Zn content in the whole body of rainbow trout larvae than ZnO-NP and organic Zn. Dekani et al. (2019) found that highest and lowest levels of zinc were detected in liver and muscles, respectively, irrespective to Zn forms. They also reported that lower concentrations of ZnO-NPs (30 and 100 mg/kg diet) relatively displayed the highest Zn ability to pass through intestine epithelium and accumulate it with highest levels in all fish tissues as compared to the respective concentrations of $ZnSO_4$ and Zn-proteinate. This discrepancy could be attributed to different chemical mechanisms of each Zn form to translocate Zn into target fish organs.

5. Conclusion

The biogenic ZnO-NP using ginger (*Z. officinale*) extract or ZnO-K are beneficial for Nile tilapia as growth promoting, modulating the digestive enzymes, serum biochemical response, immunity, antioxidant enzymes capacity and related gene expression due to the high Zn availability than mineral $ZnSO_4$ form. Further studies are needed for exploring the expression of genes to clear the mode of action of ZnO-K on the health statues of fish.

Data availability statement

Data of the present article are not available.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of fish were followed by the authors.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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References

Abdel-Khalek, A.A., Kadry, M., Hamed, A., Marie, M.-A., 2015. Ecotoxicologic al impacts of zinc metal in comparison to its nanoparticles in Nile tilapia; *Oreochromis niloticus*. Journal of Basic and Applied Zoology 72, 113–125.

Abdel-Latif, H.M.R., Abdel-Tawwab, M., Khafaga, A., Dawood, M.A.O., 2020. Dietary oregano essential oil improved antioxidative status, immune-related genes, and resistance of common carp (*Cyprinus carpio* L.) to *Aeromonas hydrophila* infection. Fish and Shellfish Immunology 104, 1–7.

Abdel-Tawwab, M., 2016. Effect of feed availability on susceptibility of Nile tilapia, *Oreochromis niloticus* (L.) to environmental zinc toxicity: growth performance, biochemical response, and zinc bioaccumulation. Aquaculture 464, 309–315.

Abdel-Tawwab, M., Samir, F., Abd El-Naby, A.S., Monier, M.N., 2018. Antioxidative and immunostimulatory effect of dietary cinnamon nanoparticles on the performance of Nile tilapia, *Oreochromis niloticus* (L.) and its susceptibility to hypoxia stress and *Aeromonas hydrophila* infection. Fish Shellfish Immunol. 78, 346–354.

Abdel-Tawwab, M., Abdel-Razek, N., Abdel-Rahman, A.M., 2019. Immunostimulatory effect of dietary chitosan nanoparticles on growth performance of Nile tilapia, *Oreochromis niloticus* (L.). Fish Shellfish Immunol. 88, 254–258.

Abdel-Tawwab, M., Khalifa, E., Diab, A.M., Khallaf, M.A., Abdel-Razek, N., Khalil, R.H., 2020a. Dietary garlic and chitosan alleviated zearalenone toxic effects on performance, immunity, and challenge of European sea bass, *Dicentrarchus labrax*, to *Vibrio alginolyticus* infection. Aquac. Int. 28, 493–510.

Abdel-Tawwab, M., Monier, M.N., Abdelrhman, A.M., Dawood, M.A.O., 2020b. Effect of dietary multi-stimulants blend supplementation on performance, digestive enzymes, and antioxidants biomarkers of common carp, *Cyprinus carpio* L. and its resistance to ammonia toxicity. Aquaculture 528, 735529.

Aebi, H., 1984. Catalase in vitro, Methods in Enzymology. 1984. Elsevier, pp. 121–126.

Aguzzi, C., Cerezo, P., Viseras, C., Caramella, C., 2007. Use of clays as drug delivery systems: possibilities and limitations. Appl. Clay Sci. 36, 22–36.

Akrami, R., Gharaei, A., Mansour, M.R., Galeshi, A., 2015. Effects of dietary onion (*Allium cepa*) powder on growth, innate immune response and hemato-biochemical parameters of beluga (*Huso huso* Linnaeus, 1754) juvenile. Fish Shellfish Immunol. 45, 828–834.

Albengres, E., Urien, S., Tillement, J.P., Oury, P., Decourt, S., Flouvat, B., Drieu, K., 1985. Interactions between smectite, a mucus stabilizer, and acidic and basic drugs. Eur. J. Clin. Pharmacol. 28, 601–605.

Albrecht, M.A., Evans, C.W., Raston, C.L., 2006. Green chemistry and the health implications of nanoparticles. Green Chem. 8, 417–432.

Aliko, V., Qirjo, M., Sula, E., Morina, V., Faggio, C., 2018. Antioxidant defense system, immune response and erythron profile modulation in gold fish, *Carassius auratus*, after acute manganese treatment. Fish Shellfish Immunol. 76, 101–109.

Alishahi, A., Mirvaghefi, A., Tehrani, M., Farahmand, H., Koshio, S., Dorkoosh, F., Elsabee, M.Z., 2011. Chitosan nanoparticle to carry vitamin C through the gastrointestinal tract and induce the non-specific immunity system of rainbow trout (*Oncorhynchus mykiss*). Carbohydr. Polym. 86, 142–146.

Anand Raj, L.F.A., Jayalakshmy, E., 2015. Biosynthesis and characterization of zinc oxide nanoparticles using root extract of *Zingiber officinale*. Orient. J. Chem. 31 (1), 51–56.

AOAC, 2005. Official Methods of Analysis. Association of Official Analytical Chemists, Arlington,VA.

Asaikkutti, A., Bhavana, P.S., Vimalab, K., Karthika, M., Cheruparambathc, P., 2016. Dietary supplementation of green synthesized manganese-oxide nanoparticles and its effect on growth performance, muscle composition and digestive enzyme activities of the giant freshwater prawn *Macrobrachium rosenbergii*. J. Trace Elem. Med. Biol. 35, 7–17.

Ashmead, H.D., 1992. The Roles of Amino Acid Chelates in Animal Nutrition. Noyes Publications, Park Ridge NJ 479 pp.

Awad, A., Zaglool, A.W., Ahmed, S.A., Khalil, S.R., 2019. Transcriptomic profile change, immunological response and disease resistance of *Oreochromis niloticus* fed with conventional and Nano-zinc oxide dietary supplements. Fish Shellfish Immunol. 93, 336–343.

Bancroft, O.D., Stevens, A., 2010. Theory and Practice of Histological Technique. Chirchil Livingstone, Edinburgh, London and New York.

Beck, B.H., Barnett, M., Farmer, B.D., Peatman, E., Carter, D., 2015. Kaolinitic clay protect against *Flavobacterium columnare* infection in channel catfish *Ictalurus punctatus* (Rafinesque). J. Fish Dis. 38, 241–248.

Benzie, I.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. Anal. Biochem. 239, 70–76.

Bernfeld, P., 1951. Amylases α and β. In: Colowick, P., Kaplan, N.O. (Eds.), Methods in Enzymology. vol. 1. Academic Press, New York, NY, pp. 149–157.

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., Kalayci, O., 2012. Oxidative stress and antioxidant defense. World Allergy Organ J. 5, 9.

Boyd, C.E., Tucker, C.S., 2012. Pond Aquaculture Water Quality Management. Kluwer Academic Publishers, Boston, MA, USA.

Buentello, J.A., Goff, J.B., Gatlin, D.M., 2009. Dietary zinc requirement of hybrid striped bass, *Morone chrysops* × *Morone saxatilis*, and bioavailability of two chemically different zinc compounds. J. World Aquacult. Soc. 40, 687–694.

Capillo, G., Silvestro, S., Sanfilippo, M., Fiorino, E., Giangrosso, G., Ferrantelli, V., Vazzana, I., Faggio, C., 2018. Assessment of electrolytes and metals profile of the Faro Lake (capo Peloro lagoon, Sicily, Italy) and its impact on *Mytilus galloprovincialis*. Chem. Biodivers. 15 (5), 1800044.

Chupani, L., Zusková, E., Niksirat, H., Panáček, A., Lünsmann, V., Haange, S.-B., Bergen, M.V., Jehmlich, N., 2017. Effects of chronic dietary exposure of zinc oxide nanoparticles on the serum protein profile of juvenile common carp (*Cyprinus carpio* L.). Sci. Total Environ. 579, 1504–1511.

Dawood, M.A.O., Koshio, S., Ishikawa, M., Yokoyama, S., 2015. Interaction effects of dietary supplementation of heat-killed *Lactobacillus plantarum* and β-glucan on growth performance, digestibility and immune response of juvenile red sea bream,*Pagrus major*. Fish Shellfish Immunol. 45, 33–42.

de Lemos, Marina Jorge, Calixto, Lígia Fátima Lima, dos Santos Alves, Osvanira, de Souza, Daniele Santos, Reis, Bárbara Brandt MouraI Túlio Leite, 2015. Kaolin in the diet and its effects on performance, litter moisture and intestinal morphology of broiler chickens. Ciência Rural, Santa Maria 45 (10), 1835–1840.

Deer, W.A., Howie, R.A., Zussman, J., 1992. An Introduction to Rock Forming Minerals. (Harlow longman).

Dekani, L., Johari, S.A., Joo, H.S., 2019. Comparative toxicity of organic, inorganic and nanoparticulate zinc following dietary exposure to common carp (*Cyprinus carpio*). Sci. Total Environ. 656, 1191–1198.

Divyagnaneswari, M., Christybapita, R.D., 2007. Enhancement of nonspecific immunity and disease resistance in *Oreochromis mossambicus* by *Solanum trilobatum* leaf

fractions. Fish Shellfish Immunol. 23, 249–259.
Dominy, N.J., Davoust, E., Minekus, M., 2004. Adaptive function of soil consumption: an in vitro study modeling the human stomach and small intestine. J. Exp.Biol. 207, 319–324.
Doumas, B.T., Bayse, D.D., Richard, J., Carter, R.J., Peters, J.T., Robert Schaffer, R., 1981. A candidate reference method for determination of total protein in serum I. development and validation. Clin. Chem. 27 (10), 1642–1650.
Dube, A., Nicolazzo, J.A., Larson, I., 2010. Chitosan nanoparticles enhance the intestinal absorption of the green tea catechins (+) - catechin and (−) -epigallocatechin gallate. Eur. J. Pharm. Sci. 41, 219–225.
Eckerich, C., Fackelmayer, F.O., Knippers, R., 2001. Zinc affects the conformation of nucleoprotein elements formed by replication protein a (RPA) and long natural DNA molecules. Biochim. Biophys. Acta 1538, 67–75.
Eid, A.E., Ghonim, S.I., 1994. Dietary zinc requirement of fingerling *Oreochromis niloticus*. Aquaculture 119, 259–264.
Eide, D.J., 2006. Zinc transporters and the cellular trafficking of zinc. Biochim. Biophys. Acta 1763, 711–722.
Einarsson, S., Davies, P.S., Talbot, C., 1997. Effect of exogenous cholecystokinin on the discharge of the gallbladder and the secretion of trypsin and chymotrypsin from the pancreas of the Atlantic salmon, *Salmo salar* L. Comp. Biochem. Physiol. (part C) 117 (1), 63–67.
El-Sayed, A.-F.M., 2019. Tilapia Culture, 2nd edition. Academic Press, Elsevier Science Publishing Co Inc, San Diego, USA.
Estaki, M., DeCoffe, D., Gibson, D.L., 2014. Interplay between intestinal alkaline phosphatase, diet, gut microbes and immunity. World J. Gastroenterol. 20, 15650.
Faggio, C., Piccione, G., Marafioti, S., Arfuso, F., Trischitta, F., Fortino, G., Fazio, F., 2014. Monthly variations of haematological parameters of *Sparus aurata* and *Dicentrarchus labrax* reared in Mediterranean land off-shore tanks. Cah. Biol. Mar. 55, 437–443.
Faiz, H., Zuberi, A., Nazir, S., Rauf, M., 2015. Zinc oxide, zinc sulfate and zinc oxide nanoparticles as source of dietary zinc: comparative effects on growth and hematological indices of juvenile grass carp (*Ctenopharyngodon idella*). Int. J. Agric. Biol. 17, 568–574.
Gatlin III, D.M., O'Connell, J.P., Scarpa, J., 1991. Dietary zinc requirement of the red drum, *Sciaenops ocellatus*. Aquaculture 92, 259–265.
Gawade, V.V., Gavade, N.L., Shinde, H.M., Babar, S.B., Kadam, A.N., Garadkar, K.M., 2017. Green synthesis of ZnO nanoparticles by using *Calotropis procera* leaves for the photodegradation of methyl orange. J. Mater. Sci.: Mater Electron 28, 14033–14039.
Gharaei, A., Ghaffari, M., Keyvanshokooh, S., Akrami, R., 2011. Changes in metabolic enzymes, cortisol and glucose concentrations of Beluga (*Huso huso*) exposed to dietary methylmercury. Fish Physiol. Biochem. 37, 485–493.
Gharaei, A., Khajeh, M., Khosravanizadeh, A., Mirdar, J., Fadai, R., 2020. Fluctuation of biochemical, immunological, and antioxidant biomarkers in the blood of beluga (*Huso huso*) under effect of dietary ZnO and chitosan–ZnO NPs. Fish Physiol. Biochem. 46, 547–561.
Girardeau, J.P., 1987. Smectite aggregation by *Escherichia coli*. Acta Gastroenterol. Belg. 50, 181–192.
Gopal, V., Parvathy, S., Balasubramanian, P.R., 1997. Effect of heavy metals on the blood protein biochemistry of the fish *Cyprinus carpio* and its use as a bio-indicator of pollution stress. Environ. Monit. Assess. 48 (2), 117–124.
Halliwell, B., Gutteridge, J.M., 2015. Free Radicals in Biology and Medicine. Oxford University Press, USA.
Handy, R.D., 2012. Nanotechnology in fisheries and aquaculture. In: Fisheries Society of the British Isles School of Biomedical and Biological Sciences. University of Plymouth, Drake Circus, Plymouth. UK.
Harikrishnana, R., Jawaharb, S., Srikanthanb, C., Parayc, B.A., Al-Sadoonc, M.K., Balasundaramd, C., 2018. Kaolin incorporated diet on growth and immune response in *Ctenopharyngodon idellus* against *Aeromonas hydrophila*. Fish Shellfish Immunol. 77, 364–373.
Hassaan, M.S., Mohammady, E.Y., Soaudy, et al., 2019. Effect of *Silybum marianum* seeds as a feed additive on growth performance, serum biochemical indices, antioxidant status, and gene expression of Nile tilapia, *Oreochromis niloticus* (L.) fingerling. Aquaculture 509, 178–187.
Hassaan, M.S., Mohammady, E.Y., Soaudy, M.R., Palma, J., Shawer, E.E., El-Haroun, E., 2020a. The effect of dietary sericite on growth performance, digestive enzymes activity, gut microbiota and haematological parameters of Nile tilapia, *Oreochromis niloticus* (L.) fingerlings. Anim. Feed Science Technol. https://doi.org/10.1016/j.anifeedsci.2020.114400.
Hassaan, M.S., Nssar, K.M., Mohammady, E.Y., Amin, A., Tayel, S.I., El-Haroun, E.R., 2020b. Nano-zeolite efficiency to mitigate the aflatoxin B1 (AFB1) toxicity: effects on growth, digestive enzymes, antioxidant, DNA damage and bioaccumulation of AFB1 residues in Nile tilapia (*Oreochromis niloticus*). Aquaculture 523, 735123.
Hossain, M.A., Matsui, S., Furuichi, M., 2003. Effect of zinc and manganese supplementation to tricalcium phosphate rich diet for tiger puffer (*Takifugu rubripes*). Bangladesh J. Fish. Res. 7 (2), 189–192.
Hrenovic, J., Milenkovic, J., Daneu, N., Kepcija, R.M., Rajic, N., 2012. Antimicrobial activity of metal oxide nanoparticles supported onto natural clinoptilolite. Chemosphere 88, 1103–1107.
Hu, C.H., Xu, Y., Xia, M.S., Xiong, L., Xu, Z.R., 2008. Effects of Cu2+ −exchanged montmorillonite on intestinal microflora, digestibility and digestive enzyme activities of Nile tilapia. Aquac. Nutr. 14, 281–288.
Hu, C.H., Gu, L.Y., Luan, Z.S., Song, J., Zhu, K., 2012. Effects of montmorillonite–zinc oxide hybrid on performance, diarrhea, intestinal permeability and morphology of weanling pigs. Anim. Feed Sci. Technol. 177 (1–2), 108–115.
Hu, C.H., Qian, Z.C., Song, J., Luan, Z.S., Zuo, A.Y., 2013. Effects of zinc oxide-montmorillonite hybrid on growth performance, intestinal structure, and function of broiler chicken. Poult. Sci. 92 (1), 143–150.
Hu, C.H., Xiao, K., Jiao, L.F., Song, J., 2014. Effects of zinc oxide supported on zeolite on growth performance, intestinal barrier function and digestive enzyme activities of Nile tilapia. Aquacult. Nut. 20, 486–493.
Huang, S., Wang, L., Liu, L., Hou, Y., Li, L., 2015. Nanotechnology in agriculture, livestock, and aquaculture in China, a review. Agron. Sustain. Dev. 35 (2), 369–400.
Hummel, B.C.W., 1959. A modified spectrophotometric determination of chymotrypsin, trypsin and thrombin. Canad. J. Biochem. Physiol. 37, 1393–1399.
Ipsa, S., Nayak, P.L., 2013. Antimicrobial activity of copper nanoparticles synthesised by ginger (*Zingiber officinale*) extract. World J. Nano Scie. Technol. 1, 10–13.
Jafarinejad, R., Gharaei, A., Mirdar Harijani, J., 2018. Dietary ginger improve growth performance, blood parameters, antioxidant capacity and gene expression in *Cyprinus carpio*. Iran. J. Fish. Sci. https://doi.org/10.22092/ijfs.2018.119876.
Ji, W., Ping, H.C., Wei, K.J., Zhang, G.R., Shi, Z.C., Yang, R.B., et al., 2015. Ghrelin, neuropeptide Y (NPY) and cholecystokinin (CCK) in blunt snout bream (*Megalobrama amblycephala*): cDNA cloning, tissue distribution and mRNA expression changes responding to fasting and refeeding. Gen. Comp. Endocrinol. 223, 108–119.
Kaya, H., Aydın, F., Gürkan, M., Yılmaz, S., Ates, M., Demir, V., Arslan, Z., 2016. A comparative toxicity study between small and large size zinc oxide nanoparticles in tilapia (*Oreochromis niloticus*): organ pathologies, osmoregulatory responses and immunological parameters. Chemosphere 144, 571–582.
Khosravi-Katuli, K., Lofrano, G., Pak Nezhad, H., et al., 2018. Effects of ZnO nanoparticles in the Caspian roach (*Rutilus rutilus caspicus*). Sci. Total Environ. 626, 30–41.
Konturek, S.J., Zabielski, R., Konturek, J.W., Czarnecki, J., 2003. Neuroendocrinology of the pancreas; role of brain–gut axis in pancreatic secretion. Europ. J. Pharmacol. 481, 1–14.
Kutláková, K.M., Tokarský, J., Peikertov, O., 2015. Functional and eco-friendly nanocomposite kaolinite/ZnO with high photocatalytic activity. Appl. Catal. B Environ. 162, 392–400.
Kuzma, J., 2006. Nanotechnology oversight: just do it. Environ. Law. Reprod. 36, 10913–10920.
Lee, J.W., Kim, J.E., Shin, Y.J., Ryu, J.S., Eom, I.C., Lee, J.S., Kim, Y., Kim, P.J., Choi, K.H., Lee, B.C., 2014. Serum and ultrastructure responses of common carp (*Cyprinus carpio* L.) during long-term exposure to zinc oxide nanoparticles. Ecotoxicol. Environ. Saf. 104, 9–17.
Li, M.R., Huang, C.H., 2016. Effect of dietary zinc level on growth, enzyme activity and body trace elements of hybrid tilapia, *Oreochromis niloticus* × *O. aureus*, fed soya bean meal-based diets. Aquac. Nutr. 22, 1320–1327.
Liang, J.J., Yang, H.J., Liu, Y.J., Tian, L.X., Liang, G.Y., 2012. Dietary zinc requirement of juvenile grass carp (*Ctenopharyngodon idella*) based on growth and mineralization. Aquac. Nutr. 18, 380–387.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real time quantitative PCR and the $2^{-DD}C_T$ method. Methods 25, 402–408.
Lonnerdal, B., 2000. Dietary factors influencing zinc absorption. J. Nutr. 130, 1378–1383.
Maage, A., Julshamn, K., 1993. Assessment of zinc status in juvenile Atlantic salmon *Salmo salar*, By measurement of whole body and tissue levels of zinc. Aquaculture 117, 179–191.
Manduzio, H., Monsinjon, T., Galap, C., Leboulenger, F., Rocher, B., 2004. Seasonal variations in antioxidant defences in blue mussels *Mytilus edulis* collected from a polluted area: major contributions in gills of an inducible isoform of cu/Zn superoxide dismutase and of glutathione S-transferase. Aquat. Toxicol. 70, 83–93.
Maret, W., Krežel, A., 2007. Cellular zinc and redox buffering capacity of metallothionein/thionein in health and disease. Mol. Med. 13, 371–375.
Martin-Kleiner, I., Flegar-Mestric, Z., Zadro, R., Breljak, D., Stanovic, S., 2001. The effect of the zeolite clinoptilolite on serum chemistry and hematopoiesis in mice. Food Chem. Toxicol. 39, 717–727.
Maton, P., Selden, A., Chadwick, V., 1984. Differential distribution of molecular forms of cholecystokinin in human and porcine small intestinal mucosa. Regul. Pept. 8 (1), 9–19.
Micale, V., Campo, S., D'Ascola, A., Guerrera, M.C., Levanti, M.B., Germanà, A., Muglia, U., 2014. Cholecystokinin: how many functions? Observations in seabreams. Gen. Comp. Endocrinol. 205, 166–167.
Mirghaed, A.T., Ghelichpour, M., Hoseini, S.M., Amini, K., 2017. Hemolysis interference in measuring fish plasma biochemical indicators. Fish Physiol.Biochem. 43, 1143–1151.
Mirghaed, A.T., Hoseini, S.M., Ghelichpour, M., 2018. Effects of dietary 1, 8-cineole supplementation on physiological, immunological and antioxidant responses to crowding stress in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 81, 182–188.
Moazenzadeh, K., Islami, H.R., Zamini, A., Soltani, M., 2018. Effects of dietary zinc level on performance, zinc status, tissue composition and enzyme activities of juvenile Siberian sturgeon, *Acipenser baerii* (Brandt 1869). Aquac. Nutr. 24, 1330–1339.
Moin, V.M., 1986. A simple and specific method for determining glutathione peroxidase activity in erythrocytes. Lab Delo 12, 724–727.
Murray, R.K., Granner, D.K., Mayes, P.A., Rodwell, V.W., 2003. Harper's Illustrated Biochemistry, 26th edition. McGraw-Hill, Medical Publishing Division 818 p.
Nishikimi, M., Appaji Rao, N., Yagi, K., 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun. 46 (2), 849–854.
NRC, 2011. Nutrient Requirements of Fish and Shrimp. National Academy Press, Washington DC, USA.
Ogawa, D., Asanuma, M., Miyazaki, I., Tachibana, H., et al., 2011. High glucose increases metallothionein expression in renal proximal tubular epithelial cells. Exp. Diabetes Res. 2011, 534872.
Ogino, C., Yang, G.Y., 1978. Requirement of rainbow trout for dietary zinc. Bull. Jpn. Soc. Sci. Fish. 44, 1015–1018.
Ogino, C., Yang, G.Y., 1979. Requirement of carp for dietary zinc. Bull. Jpn. Soc. Sci.

Fish. 45.
Olechnowicz, J., Tinkov, A., Skalny, A., Suliburska, J., 2018. Zinc status is associated with inflammation, oxidative stress, lipid, and glucose metabolism. J. Physiol. Sci. 68, 19–31.
Onuegbu, C.U., Aggarwal, A., Singh, N.B., 2018. ZnO nanoparticles as feed supplement on growth performance of cultured african catfish fingerlings. J. Sci. Ind. Res. 77, 213–218.
Pagano, M., Porcino, C., Briglia, M., Fiorino, E., Vazzana, M., Silvestro, S., Faggio, C., 2017. The influence of exposure of cadmium chloride and zinc chloride on haemolymph and digestive gland cells from *Mytilus galloprovincialis*. Int. J. Environ. Res. 11 (2), 207–216.
Pelaseyed, T., Bergström, J.H., Gustafsson, J.K., Ermund, A., et al., 2014. The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system. Immunol. Rev. 260 (1), 8–20.
Phillips, T.D., 1999. Dietary clay in the chemoprevention of aflatoxin-induced disease. Toxicol. Sci. 52, 118–126.
Priyaa, G.H., Kumidini, B.S., 2014. Biological synthesis of silver nanoparticles using ginger (*Zingiber officinale*) extract. J. Environ. Nanotechnol. 3, 32–40.
Rahimi, M., Mobedi, H., Behnamghader, A., Nateghi Baygi, A., Mivehchi, H., Biazar, E., 2012. Fat-soluble vitamins release based on clinoptilolite zeolite as an oral drug delivery system. Lett. Drug Des. Discov. 9, 213–217.
Rawling, M.D., Merrifield, D.L., Davies, S.J., 2009. Preliminary assessment of dietary supplementation of Sangrovit® on red tilapia (*Oreochromis niloticus*) growth performance and health. Aquaculture 294, 118–122.
Ray, C.S., Singh, B., Jena, I., Behera, S., Ray, S., 2017. Low alkaline phosphatase (ALP) in adult population an indicator of zinc (Zn) and magnesium (mg) deficiency. Curr. Res. Nutr. Food Sci. J. 5, 347–352.
Reitman, A., Frankel, S., 1957. Determination of aspartat aminotansferase. Am. J. Clin.Path. 28–56.
Ruas, C.B.G., dos Santos, C.C., de Araújo, H.S.S., Espíndola, E.L.G., Fernandes, M.N., 2008. Oxidative stress biomarkers of exposure in the blood of cichlid species from a metal contaminated river. Ecotoxicol. Environ. Saf. 71, 86–93.
SÁ, M.V. Do Carmo E., Pezzato, L.E., Barros, M.M., Padilha, P. De Magalhães, 2005. Relative bioavailability of zinc in supplemental inorganic and organic sources for Nile tilapia *Oreochromis niloticus* fingerlings. Aquac. Nutr. 11, 273–281.
Sakr, S.A., Jamal, S.M.A.L., Lail, A.I., 2005. Fenvalerate induced histopathological and histochemical changes in the liver of the catfish *Clarias gariepinus*. J. Appl. Sci. Res. 1 (3), 263–267.
Santos, W.M., Costa, L.S., López-Olmeda, J.F., Costa, N.C.S., Santos, F.A.C., Oliveira, C.G., Ribeiro, P.A.P., 2020a. Dietary protein modulates digestive enzyme activities and gene expression in red tilapia juveniles. Animal 1–9.
Santos, W.M., Costa, L.S., López-Olmeda, J.F., Costa, N.C.S., Santos, F.A., Gamarano, P.G., Ribeiro, P.A., 2020b. Effects of dietary protein levels on activities of protease and expression of ingestion and protein digestion-related genes in Nile tilapia juveniles. Aquac. Res. 51 (7), 2973–2984.
SAS, 1993. Statistical Analysis System, SAS/STAT user Guide Release 6.03 Edition. SAS Institute Inc, Cary, North Carolina, USA.
Shahpar, Z., Johari, S.A., 2019. Effects of dietary organic, inorganic, and nanoparticulate zinc on rainbow trout, *Oncorhynchus mykiss* larvae. Biol. Trace Elem. Res. 190 (2), 535–540.
Sharma, P., Jha, A.B., Dubey, R.S., Pessarakli, M., 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J. Bot. 2012, 217037.
Shearer, K.D., Maage, A., Opstvedt, J., Mundheim, H., 1992. Effects of high-ash diets on growth, feed efficiency, and zinc status of juvenile Atlantic salmon (*Salmo salar*). Aquaculture 106, 345–355.
Sheehan, D.C., Hrapchak, B.B., 1980. Theory and Practice of Histotechnology. St-Louis, Toronto, USA.
Sheikh Asadi, M., Gharaei, A., Mirdar Harijani, J., Arshadi, A., 2018. A comparison between dietary effects of *Cuminum cyminum* essential oil and *Cuminum cyminum* essential oil, loaded with iron nanoparticles, on growth performance, immunity and antioxidant indicators of white leg shrimp (*Litopenaeus vannamei*). Aquacult. Nut. 24 (5), 1466–1473.
Siklar, Z., Tuna, C., Dallar, Y., Tanyer, G., 2003. Zinc deficiency: a contributing factor of short stature in growth hormone deficient children. J. Trop. Pediatr. 49, 187–188.
Silva, W.S., Costa, L.S., López-Olmeda, J.F., Costa, N.C.S., Santos, W.M., Ribeiro, P.A.P., Luz, R.K., 2019. Gene expression, enzyme activity and performance of Nile tilapia larvae fed with diets of different CP levels. Animal 13 (7), 1376–1384.
Singh, R.P., Magesh, S., Rakkiyappan, C., 2011. Ginger (*Zingiber officinale*) root extract: a source of silver nanoparticles and their application. IJBEST 02, 75–80.
Sinha, S., Pan, I., Chanda, P., Sen, S.K., 2009. Nanoparticles fabrication using ambient biological resources. J. Appl. Biosci. 19, 1113–1130.
Spry, D.J., Hodson, P.V., Wood, C.M., 1988. Relative contributions of dietary and waterborne zinc in the rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. 45, 32–41.
Strugala, V., Allen, A., Dettmar, P.W., Pearson, J.P., 2003. Colonic mucin: method of measuring mucus thickness. Proc. Nutr. Soc. 62, 237–243.
Swain, P.S., Rao, S.B.N., Rajendran, D., Dominic, G., Selvaraju, S., 2016. Nano zinc, an alternative to conventional zinc as animal feed supplement: a review. Anim. Nutr. 2 (3), 134–141.
Swain, P., Das, R., Das, A., Padhi, S.K., Das, K.C., Mishra, S.S., 2019. Effects of dietary zinc oxide and selenium nanoparticles on growth performance, immune responses and enzyme activity in rohu, *Labeo rohita* (Hamilton). Aquacult. Nut. 25, 486–494.
Tan, B., Mai, K., 2001. Zinc methionine and zinc sulfate as sources of dietary zinc for juvenile abalone, *Haliotis discus hannai* Ino. Aquaculture 192, 67–84.
Tawfik, M.M.M., Moustafa, M.M., Abumourad, I.M.K., El-Meliegy, E.M., Refai, M.K., 2017. Evaluation of Nano Zinc Oxide Feed Additive on Tilapia Growth and Immunity, 15th Intern Conf Envi Sc and Techn. (Greece).
Tietz, N., Burtis, C., Duncan, P., Ervin, K., Petitclerc, C., Rinker, A., Shuey, D., Zygowicz, E., 1983. A reference method for measurement of alkaline phosphatase activity in human serum. Clin. Chem. 29, 751–761.
Uchiyama, M., Mihara, M., 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem. 86 (1), 271–278.
Wang, K., Wang, W.X., 2015. Optimal dietary requirements of zinc in marine medaka *Oryzias melastigma*: importance of daily net flux. Aquaculture 448, 54–62.
Wang, C., Zhang, L., Su, W., Ying, Z., He, J., Zhang, L., et al., 2017. Zinc oxide nanoparticles as a substitute for zinc oxide or colistin sulfate: effects on growth, serum enzymes, zinc deposition, intestinal morphology and epithelial barrier in weaned piglets. PLoS One 12 (7), e0181136. https://doi.org/10.1371/journal.pone.0181136.
Willis, J.N., Sunda, W.G., 1984. Relative contributions of food and water in the accumulation of zinc by two species of marine fish. Mar. Biol. 80, 273–279.
Wu, C., Zhang, W., Mai, K., Xu, W., Zhong, X., 2011. Effects of dietary zinc on gene expression of antioxidant enzymes and heat shock proteins in hepatopancreas of abalone *Haliotis discus hannai*. Comp. Biochem. Physiol. C 154, 1–6.
Yildirım, Ö., Türker, A., Şenel, B., 2009. Effects of natural zeolite (Clinoptilolite) levels in fish diet on water quality, growth performance and nutrient utilization of Tilapia (*Tilapia zillii*) fry. Environ. Bull. 18, 1567–1571.
Yuan, J.S., Reed, A., Chen, F., Stewart, C.N., 2006. Statistical analysis of real-time PCR data. BMC Bioinforma. 7, 85. https://doi.org/10.1186/1471-2105-7-85.
Zamani, A., Hajimoradloo, A., Madani, R., Farhangi, M., 2009. Assessment of digestive enzymes activity during the fry development of the endangered Caspian brown trout *Salmo caspius*. J. Fish Biol. 75 (4), 932–937.
Zhang, Y., Xu, C., He, Y., Wang, X., Xing, F., Qiu, H., Liu, Y., Ma, D., Lin, T., Gao, J., 2011. Zeolite/polymer composite hollow microspheres containing antibiotics and the in vitro drug release. J. Biomater. Sci. Polym. 22, 809–822.
Zhou, J., Xu, N., Wang, Z.L., 2006. Dissolving behaviour and stability of ZnO nano wires in biofluids: a study on biodegradability and biocompatibility of ZnO nanostructures. Adv. Mater. 18, 2432–2435.