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SHORT COMMUNICATION

The improvement of growth, immunological and hematological parameters of juvenile common carp (*Cyprinus carpio*) treated with zinc sulfate and vitamin E nanoparticles

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Abstract The mutual effects of zinc sulfate nanoparticles, and vitamin E nanoparticles on growth, immunological, and the hematological parameters of fingerling juvenile common carp (*Cyprinus carpio*) with an average weight of 9.64 ± 0.34 g were evaluated for 60 days using four different treatments (D0, diet without any zinc or vitamin E supplements, and three diets (D1, D2, D3) supplemented with normal vitamin E, zinc sulfate, vitamin E nanoparticles, and zinc sulfate nanoparticles respectively). The results showed that after 60 days of experimental feeding, in the treatment group D3, the percent weight gain (% WG, 144.56 ± 1.85), specific growth rate (SGR, 1.46 ± 0.13) and feed conversion ratio (FCR, 4.59 ± 0.68) were significantly improved compared to other groups ($P < 0.05$). Furthermore, the levels of total serum protein, lysozyme, immunoglobin M (IgM), cholesterol, hemoglobin, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) in the treatment groups D2 and D3 were higher than other treatments ($P < 0.05$). White blood cells and Lymphocytes were higher in the treatment D1, D2 and D3 than treatment D0 ($P < 0.05$). The lowest level of cortisol, glucose, HIS and ALT belonged to the same groups ($P < 0.05$). The majority of the growth, immunological, and hematological parameters rose in treatment group D1 as compared to the control group, however the disparities between these groups were more pronounced. Hence, the findings demonstrate that fingerling common carp have a critical need for zinc and vitamin E, either in their natural forms or as nanoparticles.

Keywords Common carp . Growth . Nanoparticles . Zinc sulfate . Vitamin E

Introduction

Aquaculture is one of main strategies for the production of proteins which has gained a lot of attraction in terms of its advantages over other methods (Ghanbary et al. 2021). In the past, the marine resources were mainly supplied by fishing, but in the past few decades, aquaculture has accounted for more than half of total global fish consumption (Naderi et al. 2017). In order to enhance the tolerance of marine organisms to dense conditions during breeding and proliferation, as well as to the environmental stress that fish are exposed to, food supplements play a crucial role in nutrition and production. Therefore, the enrichment of diet using the vitamins and minerals is supposed to be an effective strategy for enhancing the growth rate, immunity, and resistance to diseases (Khan et al. 2017).

Zinc is one of important trace elements in fish which has an important role in biological, physiological, and reproductive processes, such as biosynthesis of enzymes, hormones, proteins, and carbohydrates (Wang et al. 2015). The activities of more than 300 different enzymes are directly dependent on the pres-

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ence of zinc (Chen et al. 2015; Swain et al. 2016). Maintaining normal levels of zinc is very important in physiology, growth, increasing the production yield, and bone mineralization of fish whereas the excess of zinc not only results in excessive excretion, but also was shown to have a negative effect on bone mineralization (Liang et al. 2012).

Moreover, using the compounds containing zinc in oral form reduces their efficacy, stability, digestion, absorption, and dissolution (Thilakarathna et al. 2013; Watkins et al. 2015). Consequently, researchers have sought to reduce zinc consumption while increasing its bioavailability (Swain et al. 2016).In this regard, nanotechnology has helped solve the problems related to processing, storage, and consumption of food additives by providing nanoparticles in the size range from 10-100 nm (Liu et al. 2009; Kettel et al. 2011). Zinc nanoparticles have unique properties, such as high gastrointestinal absorption, high bioavailability, enhanced bactericidal activity, and degradation (Albrecht et al. 2006; Dube et al. 2010). The shape, size, optical properties, and electrical properties of materials are related to their physicochemical properties. Reducing the size of macromolecules to the nanoscale changes these properties, and expands their applications (Alishahi et al. 2011; Rather et al. 2011). The absorption, and bioavailability of zinc in the diet can be affected by the natural, and chemical form of zinc in the diet, the composition of dietary proteins, and the presence of phthalate and tricalcium phosphate in the diet (Lonnerdal 2000; Tan et al. 2001).

Vitamin E plays a key role in many physiological, and biochemical processes, such as growth, reproduction, and immune responses (Zhou et al. 2013). It is an essential vitamin for humans and animals; however, the fish body cannot synthesize it (Peng et al. 2009). A large body of research was carried out on the effects of zinc nanoparticles and zinc oxide nanoparticles as dietary supplements on growth parameters, hematological parameters and immunity of fish (Faiz et al. 2015; Taheri et al. 2017; Kumar et al. 2018; Khan et al. 2020; Thangapandiyan et al. 2020). Similarly there is considerable literature on the protective effects of vitamin E on the exposure of fish to zinc nanoparticles (Farsani et al. 2017), effects of zinc oxide nanoparticles on immunity, and resistance to diseases (Awad et al. 2019).

The common carp (*Cyprinus carpio*) is one of commonly cultivated species of fish worldwide which has ranked 7th among all fish species in 2016 with an annual production rate of 3’000’000 tons (Pauly et al. 2017). The present study is aimed at studying the mutual effect of vitamin E nanoparticles (Vit E NPs) and zinc sulfate nanoparticles (Zn NPs) on growth, immunity and hematological parameters of Common Carp. To the best of our knowledge, there is only one study on the protective effect of vitamin E in Nile tilapia fish exposed to zinc oxide nanoparticles, and the present study is the first study on the mutual effects of Vit E NPs, and Zn NPs on the growth, survival and physiological parameters of Common Carp or any other marine organisms. Hence, the results from this study can add to available literature data, and serve as a guide for any future studies on the counter effects of Zn NPs, and Vit E NPs on growth, immunity and hematological parameters of Common Carp.

Materials and methods

The present study was carried out as a controlled randomized trial over a period of 60 days from October to November 2020 in the research center of Iranian Fisheries organization in Ghasre Shirin, Kermanshah Province, and West of Iran. A total of 360 fingerling Common Carps with an average weight of 9.64 ± 0.34 g were obtained from a reliable farm (Ghasre Shirin, Kermanshah Province) and transferred to a warm-water fish farming facility (Ghasre Shirin Fisheries Research Center, Kermanshah, Iran). After one week of quarantine, fish were fed for two weeks with the basic fish feed committee (FFC) diet (K. danehtalaee company, Arak, Iran) containing 35-38 % raw protein, 4-8 % raw fat, 7-11 % fiber, 7-11 % raw ash, 5-11 % moisture, and 1-1.5 % phosphorous. After two weeks, fish were randomly divided over 12 fiberglass reservoirs (3 per treatment group). All reservoirs contained the same number of fish, and the same conditions in terms of total volume (300 liters), and qualitative properties of water. The water temperature, pH and dissolved oxygen were kept constant at 25.4 C°, 7.8, and 6.9 mg/L, respectively throughout the study. Physicochemical properties of water were measured with a portable monitoring device (Aqua combo, China).

Preparation of zinc sulfate and vitamin E nanoparticles

The vitamin E and zinc sulfate came from an animal food manufacturer in the Iranian city of Ravansar,

which is in the province of Kermanshah. The nanomaterials were produced in Caspian Sea ecology research center (CSERC), (Sari, Iran) with green method with a particle size of 50-70 nm. Particle size analysis was performed using scanning electron microscope (SEM) particle analyzer, and after confirming the size, zinc, and vitamin E nanoparticles were used.

Preparation of the diets and feeding the fish

The diets used in the study consisted of a basic Finger linger Feed Carp or FFC diet (D0) without the addition of any vitamin E or zinc, D1 containing 100 IU vitamin E and 15 mg/kg zinc sulfate, D2 containing 100 IU vitamin E nanoparticles and 15 mg/kg zinc sulfate nanoparticles, and D3 containing 300 IU vitamin E nanoparticles and 15 mg/kg zinc sulfate nanoparticles. The concentrations of zinc (15 mg), and vitamin E (100 IU or 300 IU) were chosen according to the literature data (Barrows et al. 2007). Normal vitamin E was purified to 50% purity, and normal zinc sulfate was purified to 35% purity. Vitamin E nanoparticles were prepared as a surfactant with 60 % purity, and a total of 2 mL was added to the diet using a syringe. The required amounts of vitamin E and zinc sulfate nanoparticles were mixed with fish oil (20 mL/kg diet), and were then sprayed over the different diets (Nootash et al. 2013). To D1 diets, the normal oil without any additives was added. The fish were fed with the assigned diet for 60 days (3 times per day at 8, 13 and 19 h in the first 40 days and twice per day at 8 and 16 h for the rest of the period). The water in the reservoirs was flushed on a daily basis.

Measurement of growth parameters

To evaluate the effects of different amounts of vitamin E NPs, and zinc sulfate NPs, and compare the different treatment groups, the fish were weighed at the beginning and end of the study using a scale with a precision level of 0.01 g and their total length (head to tail) was measured with a ruler with a precision level of 1 mm (Hung et al. 1989). The growth parameters were calculated using following equations (Hevrøy et al. 2005; Helland et al. 1996):

$$\text{Weight gain (WG)} = \text{Average final weight-Average initial weight}$$

$$\%\ \text{Weight gain (WG \%)} = \frac{(\text{final weight(g)} - \text{initial weight(g)}) \times 100}{\text{initial weight(g)}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Amount of food consumed (g)}}{\text{Increase in body weight (g)}}$$

$$\text{Specific growth rate (SGR)} = \left(\frac{\ln \text{Final weight} - \ln \text{Initial weight}}{\text{number of days}}\right) \times 100$$

$$\text{Condition factor (CF)} = \frac{\text{Weight (g)} \times 100}{\text{Length (cm)}^3}$$

Measuring the hematological and immunological parameters

At the end of study, 5 fish were randomly selected from each group (in total 15 fish of each treatment), and were anesthetized using 100 mg/L Clove. Blood samples were obtained from their caudal vein using a 2 mm plastic syringe and needle No. 21. The fish were starved for 24 h prior to sampling. An aliquot of each sample was transferred to test tubes containing heparin (500 U/mL) and kept at 4 °C until further analysis (Mehrabi et al. 2019). The remaining blood samples were transferred to the test tubes without any heparin and allowed to sediment at room temperature for 1 h, then stored at 4 °C for 4 h. Subsequently, the samples were centrifuged for 10 min at 5000 rpm, the serum was separated and stored at -20 °C for further analysis (Heydari et al. 2020). The amount of hematocrit was measured by blood sedimentation using standard microhematocrit method and was reported as percentage (Ranzani-Paiva et al. 2004). The amount of hemoglobin was measured using standard cyanmethemoglobin method at the wavelength of 540 nm (Mehrabi et al. 2020).

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were estimated using following calculations (Verma et al. 2019):

$$(\mathrm{MCV})(\mathrm{fl}) = \frac{\text{Hematocrit} \times 10}{\text{RBC}}$$

$$(\mathrm{MCH})(\mathrm{pg}) = \frac{\text{Hemoglobin} \times 10}{\text{RBC}}$$

$$(\mathrm{MCHC})(\%) = \frac{\text{Hemoglobin} \times 100}{\text{Hematocrit}}$$

WBC differential count was performed using light microscope and hemocytometer (Borges et al. 2004).

Measuring the serum and immunological parameters

The level of immunoglobulins was determined using Elisa, and nephstar kits (Farsani et al. 2019). The serum lysozyme levels were determined by the lysis of *Micrococcus lysodicticus* method (Demers and Bayne 1997). The serum ahc50 was determined following the method by Yano et al. (Ghanbary et al. 2021). The total serum protein and serum albumin measured by colorimetric assay using the spectrophotometric kits, and the amount of globulin was calculated by subtracting the amount of albumin from the total protein (Kumar et al., 2013). The level of cortisol was measured by Eliza using IBL kits (Germany) and the serum glucose was measured using Pars Azmoun kits (Iran) (Yeh et al. 2013). The level of serum cholesterol and triglycerides were determined by enzymatic method (CHO-PAP and GPO-PAP for cholesterol and triglyceride, respectively using quantitative diagnostic kits from Pars company (Iran) at the wavelength of 510 nm (Artiss et al. 1997).

Hepatic indices and hepatic enzymes

To determine the hepatic factors, the fish liver was extracted and carefully weighed, and the following equation was used (Zaki 2007):

$$\text{Hepato smatic index (HSI)}(\%) = \frac{\text{liver weight(g)} \times 100}{\text{body weight(g)}}$$

The hepatic enzymes' function tests were performed on the plasma samples from different treatment groups using biochemical test kits from Pars Azmoon company (Iran) following the method suggested by the international federation for clinical chemistry (IFCC) using the autoanalyzer device (Cobas Mira) (Rashidian et al. 2022).

Data analysis

The statistical analysis of the data was performed using SPSS 17. Comparison among different treatment groups was made using one-way analysis of variance (ANOVA), and Duncan's multiple range test at 1% significance level. Kolmogorov-Smirnov test was performed to determine the normal distribution of data (Ghosi Mobaraki et al. 2020).

Results and discussion

After 60 days of feeding fish with experimental diets, 300 IU vitamin E NPs and 15 mg/kg zinc sulfate NPs lead to significant increase in Fbw, Fbl, WG, SGR and significant decrease in FCR of common carp *(C. carpio)* compared to other treatment ($P < 0.05$) (Table 1). No significant differences were found in the CF values.

In a study by Tawfik et al. (2017), showed a gradual increase in the weight of Nile tilapia by increasing concentration of zinc oxide in normal form and in the form of nanoparticles, but diet enrichment with zinc oxide nanoparticles resulted in greater increase in SGR compared to normal zinc oxide which was up to two

times higher in some cases (Tawfik et al. 2017). In general, in low concentrations of zinc oxide nanoparticles (15 mg/kg food) the SGR was similar to higher concentrations of normal zinc (60 mg/kg food), and high concentration of zinc oxide nanoparticles (60 mg/kg food) had the highest SGR which was 4 times higher than untreated controls. The presence of zinc in the form of nanoparticles in the diet can improve its taste and can facilitate the tissue distribution and cellular uptake of zinc leading to higher gastrointestinal absorption, and higher bioavailability (Onuegbu et al. 2018).

The changes to hematological parameters in response to different tested diets are provided in Table 2. The levels of Hb, MCV, MCH, and MCHC in fish fed with diets D3 and D4 were significantly higher than other diets ($P < 0.05$). The amount of HCT in the fish receiving 300 IU Vit E NPs and 15 mg/kg zinc sulfate was considerably higher than the other groups ($P < 0.05$).

An increase in the concentration of RBC in D3 was observed compared to the other groups however the difference was not statistically significant. Furthermore, WBC, and lymphocyte counts were considerably higher than other groups in the fish fed with D2 and D3 diets respectively ($P < 0.05$, Table 3). However, no significant difference was found in the neutrophil, eosinophil and monocyte counts among different diet groups ($P > 0.05$, Table 3).

One of the suitable efficient means of evaluating the health of fish, and the quality of the oxygenation system is to assess the hematological parameters and the changes to HCT, RBC and Hb levels (Yilmaz 2019). In the study by Faiz et al., the levels of RBC and MCHC were improved in *C. carpio* treated with 30 mg/L zinc oxide nanoparticles (Faiz et al. 2015). The results of a study on *Acipenser baerii* showed that the RBC count, Hb, and HCT were increased by increasing the amount of zinc in the fish diet (Moazenzadeh et al. 2017). Moreover, exposure to Iron and Vit C led to an increase in MCV and MCH in *Oncorhynchus mykiss* after 96 h (Adel and Khara 2017). This increase indicates the improvement in oxygen transfer capacity from the gills to the other tissues, and in the blood rheology and hemodynamics (Moazenzadeh et al. 2017). The presence of elements, such as potassium, iron, calcium, manganese, magnesium, selenium and zinc in the fish diet can increase their blood cells count (Ellis 2001).

Increase in WBC, and neutrophil counts following exposure to copper oxide nanoparticles was reported in the literature for juvenile Bluga, and rainbow trout (Khabbazi et al. 2015; Moeinnejad et al. 2019). Moazenzadeh et al. reported that using different amounts of zinc supplement in the diet of *Acipenser baerii* leads to a considerable increase in leukocytes (Moazenzadeh et al. 2017). In an 8-week study by Lin and Shiau

Table 1 Changes to growth parameters of common carp fed with different diets

	Ibw (g)	Ibl (cm)	Fbw (g)	Fbl (cm)	WG (g)	%WG	FCR	CF	SGR	S (%)
D0	9.65± 0.07 ^{a}	8.91± 0.20 ^{a}	21.30± 0.93 ^{ab}	10.35± 0.31 ^{a}	11.69± 1.06 ^{a}	124.42± 1.09 ^{a}	5.20± 0.35 ^{ab}	1.92± .09 ^{a}	1.34± 0.12 ^{a}	88
D1	9.75± 0.51 ^{a}	9.01± 0.93 ^{a}	21.36± 1.42 ^{ab}	10.73± 0.21 ^{ab}	11.52± 1.14 ^{a}	122.10± 2.11 ^{a}	5.72± 0.89 ^{b}	1.73±. 13 ^{a}	1.29± 0.13 ^{a}	91
D2	9.37± 0.38 ^{a}	8.98± 0.36 ^{a}	20.03± 1.63 ^{a}	10.47± 0.31 ^{a}	10.86± 1.19 ^{a}	120.45± 1.68 ^{a}	5.96± 0.78 ^{b}	1.74± .12 ^{a}	1.29± 0.14 ^{a}	91
D3	9.53± 0.56 ^{a}	9.1± 0.55 ^{a}	24.04± 1.75 ^{b}	10.93± 0.21 ^{b}	14.11± 1.21 ^{b}	144.56± 1.85 ^{b}	4.59± 0.68 ^{a}	1.83±. 12 ^{a}	1.46± 0.13 ^{b}	94

Data are presented as mean ± SD. Means in each column with different superscripts show a significant difference ($P < 0.05$), and values sharing identical superscript letters are not significantly different ($P > 0.05$). Ibw: Initial body weight, Ibl: Initial body length, Fbw: Final body weight, Fbl: Final body length, WG: Weight gain, %WG: %Weight gain, FCR: Feed conversion ratio, CF: Condition factor, SGR: Specific growth rate, S: Survival.

Table 2 Hematological parameters of common carp fed with different diets

	RBC($\times 10^6$/mm ^{3})	Hb (g·dl^{-1})	HCT (%)	MCV (fl)	MCH (pg)	MCHC (%)
D0	1.64 ± 0.22 ^{a}	3.83 ± 0.45 ^{a}	30.33 ± 0.54 ^{a}	184.93 ± 5.01 ^{a}	23.35 ±1.55 ^{a}	12.62 ± 1.04 ^{a}
D1	1.65 ± 0.21 ^{a}	4.41 ± 0.11 ^{b}	30.67 ± 0.65 ^{a}	185.87 ± 5.15 ^{ab}	26.72 ± 1.31 ^{ab}	14.38 ± 0.85 ^{ab}
D2	1.62 ± 0.1 ^{a}	4.77 ± 0.09 ^{bc}	30.57 ± 0.71 ^{a}	188.7 ± 5.18 ^{b}	29.44 ± 1.05 ^{b}	15.6 ± 1.09 ^{b}
D3	1.69 ± 1.1 ^{a}	4.83 ± 0.04 ^{c}	32.05 ± 0.71 ^{b}	189.64 ± 5.54 ^{b}	28.57 ± 1.09 ^{b}	15.5 ± 0.81 ^{b}

Data are presented as mean ± SD. Means in each column with different superscripts show a significant difference ($P < 0.05$), and values sharing identical superscript letters are not significantly different ($P > 0.05$). RBC: Red blood cells, Hb: Hemoglobin, HCT: Hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration.

Table 3 Nonspecific immunity parameters of common carp fed with different diets

	WBC($\times 10^3$/mm ^{3})	Lymphocyte(%)	Neutrophil (%)	Eosinophils(%)	Monocyte (%)
D0	2.40 ± 0.46 ^{a}	59.05 ± 2.05 ^{a}	29.6 ± 4.35 ^{a}	0.71 ± 0.23 ^{a}	2.86 ± 0.85 ^{a}
D1	2.93 ± 0.74 ^{ab}	70.25 ± 4.31 ^{b}	23.65 ± 3.86 ^{a}	0.51 ± 0.15 ^{a}	3.05 ± 0.9 ^{a}
D2	3.57 ± 0.93 ^{b}	65.09 ± 3.36 ^{ab}	27.57 ± 2.36 ^{a}	0.78 ± 0.33 ^{a}	3.09 ± 0.85 ^{a}
D3	2.93 ± 0.51 ^{ab}	69.41 ± 3.33 ^{ab}	28.15 ± 3.11 ^{a}	0.49 ± 0.24 ^{a}	2.99 ± 0.85 ^{a}

Data are presented as mean ± SD. Means in each column with different superscripts show a significant difference ($P < 0.05$), and values sharing identical superscript letters are not significantly different ($P > 0.05$). WBC: White blood cells.

the effect of different amounts of vitamin E (25, 50, 100, 200, 400 mg/kg) in the diet on the immunological parameters of *Epinephelus malabaricus* was investigated. The results showed an increase in the WBC count compared to the controls (Lin and Shiau 2005). The zinc-enriched diet in Oncorhynchus mykiss resulted in a significant increase in serum lysozyme activity and total immunoglobin across all treatment groups (Gharekhani et al. 2015). The lymphocyte counts increased, and the neutrophil count did not change which could be a sign of lack of stress in the fish under study.

The results presented in Table 4 indicate that the highest levels of total protein, lysozyme, IgM, triglycerides and cholesterol belonged to the fish receiving diets D2 and D3 ($P < 0.05$). The levels of cortisol and glucose were significantly lower in the fish receiving diets D2 and D3 compared to other groups ($P < 0.05$). The levels of albumin and globulin were higher than control group in the fish receiving different forms of zinc and vitamin E ($P < 0.05$).

As a result of their role in immune response, total serum protein, albumin and globulin are indicative of good health and resistance to disease and stress (Dawood et al. 2016). The levels of albumin, and globulin in fish are affected by the innate factors, such as species, size, gender, maturity and external factors, such as environmental factors, habitat, nutrition and season (Fei et al. 2022). Fei et al. reported that supplementing the diet with zinc improves the function of the immune system, including the total serum protein in fish as it's an essential trace element for the fish, and is closely related to many biochemical processes (Fei et al. 2022).

The zinc-enriched diet resulted in a significant increase in serum lysozyme activity and total immunoglobin in all treatment groups (Gharekhani et al. 2015). It was claimed zinc is an intracellular signaling molecule, and it plays an important role in cell mediated immune functions and oxidative stress. Zinc is an anti-inflammatory agent.

The changes to the level of cortisol are aligned with the levels of blood glucose which highlights the role of cortisol to prepare the fish body for combating the induced stress (Pratap and Bonga 1990). In a study by Moeinnejad et al. the levels of cortisol, glucose and total protein significantly increased in most treatment groups indicating the adverse effects of copper nanoparticles on the health of tested fish (Moeinnejad et al. 2019). Adding mineral supplements and changing the type from mineral to nanoparticles reduced glucose levels. A similar trend was observed. Therefore, in addition to the safe levels of the nanoparticles used in the study it could be claimed that the minerals in the form of nanoparticles could play a role in reducing the stress and consequently reducing the levels of blood glucose.

In a study by Vrček et al. (2016) silver nanoparticles caused a significant decline in the levels of serum triglycerides in terms of reduced production of cholesterol resulting from the reduction in intracellular activities of the hepatic cells (Vrček et al. 2016). In a study by Bita et al., the levels of cholesterol and triglycerides showed a considerable decrease following treatment with the sublethal concentrations of silver nanoparticles (Bita et al. 2017). On the other hand, it was observed in another study that iron and copper nanoparticles were more effective than other treatments to improve total serum protein, serum albumin, serum globulin, cholesterol, triglycerides, and other antioxidants and immunological factors, as well as antibacterial activity of the serum (El Basuini et al. 2016). Increases in cholesterol levels, and triglycerides were observed in diet groups D2 and D3 either due to the presence of zinc and vitamin E in nanoparticles or due to their individual or mutual effects. Unraveling the exact reason for this requires further studies.

The data from hepatosomatic index (HSI), and specific activity of liver enzymes are provided in Table 5. The lowest HSI and ALT were observed with diets D2, and D3 ($P < 0.05$). Although the activity of ALP and AST was reduced in D3 group compared to other groups, the difference was not statistically significant

Table 4 Some immunity parameters and biochemical factors of common carp fed with different diets

	Total Protein (mg/di)	Albumin (mg/di)	Globulin (g/di)	Lysozyme (µg/ml)	IgM (mg/ml)	Glucose (mg/di)	Cortisol (ng/ml)	Triglycerides (mg/di)	Cholesterol (mg/di)
D0	3.85± 0.56 ^{a}	3.06± 0.20 ^{a}	0.51± 0.09 ^{a}	6.45± 0.25 ^{a}	1.75± 0.4 ^{a}	89.06± 3.55 ^{b}	9.59± 1.05 ^{b}	210.51± 5.01 ^{a}	139.09± 3.55 ^{a}
D1	4.87± 0.06 ^{a}	3.8± 0.05 ^{b}	0.76± 0.05 ^{b}	7.09± 0.15 ^{a}	1.98± 0.19 ^{a}	89.19± 4.67 ^{b}	9.75± 1.09 ^{b}	236.11± 5.13 ^{a}	148.55± 6.24 ^{ab}
D2	5.09± 0.38 ^{b}	3.85± 0.09 ^{b}	0.89± 0.15 ^{c}	7.36± 0.33 ^{b}	4.49± 1.17 ^{b}	70.05± 3.11 ^{a}	7.86± 1.34 ^{a}	307.2± 5.18 ^{b}	165.34± 3.02 ^{c}
D3	5.36± 0.16 ^{b}	3.4± 0.11 ^{b}	0.84± 0.1 ^{c}	7.15± 0.15 ^{b}	2.43± 0.82 ^{ab}	72.33± 2.96 ^{a}	7.05± 0.65 ^{a}	298.28± 5.05 ^{b}	156.33± 4.8 ^{b}

Data are presented as mean ± SD. Means in each column with different superscripts show a significant difference ($P < 0.05$), and values sharing identical superscript letters are not significantly different ($P > 0.05$). IgM: Immunoglobulin.

Table 5 Hepatosomatic index and specific activity of liver enzymes of common carp fed with different diets

	HSI	ALP	ALT	AST
D0	3.47 ± 0.26 [b]	2.6 ± 0.18 [a]	0.79 ± 0.09 [b]	8.9 ± 1.05 [a]
D1	3.35 ± 0.16 [ab]	2.21 ± 0.51 [a]	0.55 ± 0.11 [a]	7.69 ± 1.06 [a]
D2	3.05 ± 0.06 [a]	1.92 ± 0.09 [a]	0.5 ± 0.2 [a]	6.7 ± 1.16 [a]
D3	3.15 ± 0.05 [a]	2.09 ± 0.65 [a]	0.65 ± 0.15 [ab]	7.49 ± 1.26 [a]

Data are presented as mean ± SD. Means in each column with different superscripts show a significant difference ($P < 0.05$), and values sharing identical superscript letters are not significantly different ($P > 0.05$). HSI: Hepatosomatic; ALP: Alkaline Phosphatase; ALT: Alanine aminotransferase; AST: Aspartate Aminotransferase.

($P > 0.05$).

AST and ALT are the serum components of nonfunctional plasma enzymes which are naturally occurring in the cells in some organs including liver. The damages to the hepatocytes or changes to the permeability of their cell membranes upon exposure to nanoparticles can lead to increased levels of these enzymes in the serum (Bita et al. 2017). In a study by El Shenawy et al on Nile Tilapia in 2019, it was found that the tested amounts and particularly the optimum amount of iron nanoparticles in the diet reduced the activity of liver enzymes AST and ALT significantly compared to the controls (El-Shenawy et al. 2019). In 2018, Feng et al. claimed that using vitamin E in the diet of the *Ctenopharyngodon idellus* improves the liver function, and reduces the liver enzymes (Feng et al. 2018). They suggested that vitamin E could exert a protective effect on the liver by maintaining its morphology and function, recovery of the antioxidant activity and increasing the transcription of the antioxidant genes post-intoxication (Feng et al. 2018).

As compared to the control group, the treatment group D1 containing zinc and vitamin E showed improvement in most growth parameters, immunological parameters, and hematological parameters. However, this increase was significantly higher than the treatment group D1, and the control group in the treatment groups D2, and D3 which contained the zinc and vitamin E nanoparticles. Hence, the present study provides evidence that zinc and vitamin E either in the normal from or in the form of nanoparticles are essential requirements for juvenile common carp. However, observed benefits could not be attributed to the individual or mutual effects of zinc nanoparticles, and vitamin E nanoparticles with certainty. Considering significant improvement in most of the parameters assessed in this study especially with diets D2 and D3, it is suggested to use diet D3 to improve the growth parameters and diet D2 to improve hematological and immunological parameters for breeding the juvenile common carp.

Ethics statement This study did not require any ethical approvals.

Competing interests The authors declare that they have no competing interests

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