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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*)

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Abstract

To compare the effect of different dietary inorganic sources of zinc (Zn) i.e., zinc oxide (ZnO), zinc sulfate ($ZnSO_4$) and zinc oxide nanoparticles (ZnO-NP) on growth performance and some hematological indices of juvenile grass carp, *Ctenopharyngodon idella*, a 90 day feeding experiment under laboratory condition was executed. Six 35% protein experimental diets supplemented with dietary Zn in zinc oxide nanoparticles (ZnO-NP-I, ZnO-NP-II), oxide (ZnO-I, ZnO-II) and sulfated ($ZnSO_4$-I and $ZnSO_4$-II) forms at two levels, 30 and 60 mg kg^{-1} diet each and seventh basal diet (C) without supplementation were fed to triplicate groups of fish. Significantly higher ($P < 0.05$) percentage weight gain (% WG), specific growth rate (SGR) and feed conversion ratio (FCR) was observed in group of fish fed ZnO-NP-Idiet followed by ZnO-NP-II diet. However, retarded growth was observed in response to diets enriched with sulfated form of Zn at both levels and ZnO at higher level. Moreover, dietary Zn supplementation in oxide and sulfate forms at both concentration and nanoparticle form at higher concentration significantly decreased ($P < 0.05$) the values of hematological parameters, while, ZnO-NP at the rate of 30 mg kg^{-1} diet significantly increased the red blood cells (RBCs) count and mean corpuscular hemoglobin concentration (MCHC) value. These results clearly indicated that dietary Zn supplementation in the nanoparticles form improved the growth performance and RBCs count of juvenile grass carp, *C. idella* as compare to oxide and sulfated form of Zn. © 2015 Friends Science Publishers

Keywords: Dietary zinc; Inorganic sources; Nutrition; Hematology; *Ctenopharyngodon idella*

Introduction

The role of trace elements in biological systems has been described in several animals including fish. They are required for the normal life processes like skeletal formation, maintenance of colloidal systems, regulation of acid-base equilibrium and for biologically important compounds such as hormones and enzymes (Watanabe *et al*., 1997; Ahmad *et al.*, 2013). Mineral deficiencies can cause biochemical, structural and functional pathologies, which depend on several factors, including the duration and degree of mineral deprivation.

Zinc (Zn) is an essential trace mineral that is required for growth and metabolism of all vertebrates including fish. It is needed in more than 1000 structural, catalytic and regulatory proteins, which are important for growth, development and physiology of animals (Eide, 2006; Maret and Krężel, 2007). It is a specific cofactor of many enzymes, involved in different metabolic pathways and conformation of nucleoprotein filament (Eckerich *et al*., 2001). Besides these, it is an integral part of about 20 different metalloenzymes, like alcohol dehydrogenase, alkaline phosphatase and carbonic anhydrase (Tan and Mai, 2001). The retardation of bone growth due to deficiency of Zn also proves its importance in the growth and mineralization of bone tissues (Liang *et al*., 2012).

Freshwater fish have the ability to take Zn from both food and water, nevertheless the diet is the predominant route for the absorption of this mineral (Willis and Sunda, 1984; Spry *et al.*, 1988). However, under control condition of high water borne or low dietary Zn level, the gill showed their importance in the uptake of this mineral (Spry *et al*., 1988). In freshwater fish the uptake of Zn from water occurs mainly through gills by calcium mediated pathway (Hogstrand *et al*.,1998), while intestinal Zn uptake take place mainly by carrier mediated pathway (Glover and Hogstrand, 2002).

It is well documented that normal Zn levels in freshwater (Spry *et al*., 1988) and seawater (Willis and Sunda, 1984) are insufficient to meet the requirement of growing aquatic species. Therefore, Zn is considered as an essential nutrient in finfish feed (Wei *et al*., 1999). Dietary

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Zn requirements have been established for a number of different fish species by using zinc sulfate ($ZnSO_4$) as a dietary source and found to be between 15–30 mg kg^{-1}diet for common carp (*Cyprinus carpio*) (Ogino and Yang, 1979) and rainbow trout (*Oncorhynchus mykiss*) (Ogino and Yang, 1978), 20 mg kg^{-1} diet for channel catfish (*Ictalurus punctatus*) (NRC, 1993), 35 mg kg^{-1}diet for juvenile abalone (*Haliotis discus hannai*) (Tan and Mai, 2001), between 20–25 mg kg^{-1} diet for red drum (Gatlin *et al*., 1991). However, recommended dietary Zn for maintaining whole body and serum Zn concentrations within the physiological range in Atlantic salmon (*Salmo salar*) was found to be between 37–67 mg Zn kg^{-1} dry diet (Maage and Julshamn, 1993).

High dietary levels of Zn may negatively affect the status of other elements such as iron, copper and cadmium (Heijerick *et al*., 2002), increase the cost of feed and contribute in minerals load of aquatic environment (Wekell *et al*., 1986). It was noted that dietary Zn concentrations at level of 1000 mg kg^{-1} diet impair health of rainbow trout by decreasing the blood hematocrit and hemoglobin levels in rainbow trout (Knox *et al*., 1984). However, according to other scientists, without showing adverse effect on survival and growth performance, common carp and rainbow trout can tolerate up to 1700–1900 mg Zn per kg diet (Jeng and Sun, 1981; Wekell *et al*., 1983). Conversely, deficiency of dietary Zn has been found to impair immunological responses in rainbow trout (Kiron *et al*., 1993) and depressed growth in catfish by reducing appetite and decreasing the levels of Zn and calcium in bones (Lim *et al*., 1996). Thus, normal level of Zn is important for normal physiology, growth, efficient food utilization and bone mineralization of fish, while excessive amount not only caused severe excretion of Zn but also showed negative effect on bone mineralization (Liang *et al*., 2012).

The absorption and bioavailability of dietary Zn may be exaggerated by the nature and chemical form of Zn in diet, complexity of dietary source of protein, presence of dietary phytate and tricalcium phosphate (Lonnerdal, 2000; Tan and Mai, 2001). The inorganic forms of Zn like ZnO, $ZnSO_4$ and zinc carbonate ($ZnCO_3$) have shown to have lower intestinal absorption rate as compared to methionine-chelated zinc (Zn-Met) (Tan and Mai, 2001). Similarly, Zn-Met in comparison to $ZnSO_4$ also showed greater bioavailability of Zn (Tan and Mai, 2001). Moreover, complex diet (practical corn-soybean diet) comparative to purified crystalline amino acid base diet increased the bioavailability of Zn-Met relative to $ZnSO_4$ (Paripatananont and Lovell, 1995). However, in rainbow trout, dietary supplementation of Zn in sulfate compared to chloride and nitrate form at the same level i.e., 20 mg kg^{-1} resulted in better growth performance (Watanabe *et al*., 1997).

Recently, nanotechnology has emerged as an excellent field of technology that shows its application in various sectors including agro-food system (Kuzma, 2006), aquaculture (Defra, 2009) and aquafeed (Handy, 2012). Nanotechnology involves the synthesis of nanoscale particles that exhibit unique physiochemical properties like higher intestinal absorption, bioavailability and enhanced bactericidal and catalytic activities (Albrecht *et al*., 2006; Dube *et al*., 2010). Thus, it was observed that dietary iron in the form of nanoparticles as compared to bulk form enhanced 24 and 30% growth rate in sturgeon and young carps, respectively (ETC, 2003). Similarly, dietary selenium (Se) supplementation in nanoform relative to bulk form improved the weight gain, antioxidant enzymes status like glutathione peroxidase (GSH-Px) activity and muscles Se concentration in crucian carp (*Carassius auratus gibelio)* (Wang *et al*., 2009; Zhou, 2009).

There is no information on the bio-availability and effect of zinc oxide naoparticle (ZnO-NP) in comparison to other inorganic sources of Zn like ZnO and $ZnSO_4$ on juvenile grass carp (*Ctenopharyngodon idella*). Therefore, the objective of the study was to compare the efficiency of graded levels of dietary ZnO, $ZnSO_4$ and ZnO-NP on growth performance and hematological parameters of juvenile grass carp (*C. idella*).

Materials and Methods

Collection and Maintenance of Test Organism

After grading uniform sized, *Ctenopharyngodon idella* seed of average body weight 5.49±0.04 g were purchased from Rawal Fish Seed Hatchery, Islamabad and transported live in aerated plastic bags to the Fisheries and Aquaculture Lab. Department of Animal Sciences, Quaid-i-Azam University, Islamabad. In the laboratory, fingerlings were kept in a circular fiber tank having flow through system and were acclimatized for two weeks. During the acclimatization water quality parameters maintained in the optimum range temperature, 24.5±0.5ºC, pH, 7.5–8.11, DO, 5.5 mg L^{-1} and total ammonia, < 0.25 ppm, natural day and night photoperiod (12:12) and fish were offered 35% protein prepared feed at the rate of 4% body weight.

Synthesis of ZnO-NP

Zinc oxide nanoparticles were prepared by Co-precipitation method using zinc acetate [Zn $(O_2CCH_3)_2$], sodium hydroxide (NaOH) and Triton × 100. Briefly, 50 mM Zn acetate precursor solution was prepared by dissolving 10.97 g of Zn acetate in 500 mL of water. The mixture was shaken until zinc acetate dissolved completely, and then added 15 mL of Triton × 100. Then zinc acetate was titrated against 0.2 M NaOH with constant stirring. The fall rate of the drop from the burette was approximately 1 drop per 30 sec. After titration, the solution was heated on hot plate at 100ºC along with constant stirring for 30 min. Then, the solution was kept at ambient temperature for two days without disturbance, so that all the precipitates were settled down. The solution was filtered and then precipitates were separated. At the end of filtration process, distill water was

passed slowly over the precipitates in order to remove the residual substance, and then allowed them to dry at room temperature. After drying, they were dried in furnace at 500ºC for 5 h. Then powder was grinded, sieved and characterized by X-ray diffraction (XRD) technique.

Feed Preparation

Feed formulation is presented in Table 1. All feed ingredients were purchased from local market. The inorganic dietary sources of Zn, $ZnSO_4$ and ZnO were purchased from E. Merck D-6100 Darmstadt and Sigma-Aldrich Laborchemikalein GmbH D-30926 Seelze, respectively, while zinc oxide nanoparticles (ZnO-NP) were synthesized at NCP (National Center for Physics). All feed dry ingredients were grinded to acquire fine powder and mixed with oil. Then, semisolid paste was prepared with the addition of water and passed through a meat grinder. The formed noodles were broken manually in to small uniform size pellets. To avoid oxidation, pellets were dried at low light and room temperature and then saved in air tight jars. They were stored in a refrigerator at 4ºC. Experimental diets (ZnO-NP-1, ZnO-1 and $ZnSO_4$-1) were prepared with the addition ZnO-NP, ZnO and $ZnSO_4$ respectively at the rate 30 mg kg^{-1} diet while ZnO-NP-11, ZnO-11 and $ZnSO_4$-11 were fortified with the same Zn sources but at higher level i.e., 60 mg kg^{-1} diet. For the preparation of experimental diets, the inorganic sources of Zn were mixed in their respective diets after grinding of ingredients and before blended with oil.

Experimental Design

After an acclimatization period, healthy and uniform sized fish were selected, individually weighed by using electronic top-loading balance and evenly distributed in twenty one glass aquaria (60 × 30 × 30 cm) at a stocking density of 1.31g L^{-1}. The experimental design was randomize 3 × 2 factorial and conducted in triplicate. On the basis of difference in the supplementation of inorganic source of Zn, fish were divided into seven groups and offered their respective diets. Initially fish were offered diet at the 4% body weight and after every 15 days fish were weight and feeding was adjusted.

Sampling

At the end of feeding period, fish from each aquarium were removed, anesthetized with MS222 (60 mgL^{-1}) and weighed for determination of growth performance. Blood was drawn by puncturing of caudal vein and collected in VACUETTE® EDTA K3 tubes.

Growth Performance

The growth performance parameters like weight gain (WG), percentage weight gain (% WG), specific growth rate (SGR), Feed conversion ratio (FCR), Feed conversion efficiency (FCE %) and hepatosomatic index (HSI %) were calculated by adopting the standard formulas.

Blood Analysis

Blood samples were directly processed and complete blood profile was taken by using Hematology Analyzer. Complete blood profile gives the estimation of white blood cells (WBCs) (10^3 μL^{-1}), red blood cells (RBCs) (10_6 μL^{-1}), hemoglobin (Hb) (g dL^{-1}), Hematocrit (HCT) (%), mean corpuscular volume (MCV) (FL), mean corpuscular hemoglobin (MCH) (pg) and mean corpuscular hemoglobin concentration (MCHC) (g dL^{-1}).

Results

Size of ZnO-NP

The XRD result of ZnO-NP is shown in Fig. 1. The calculated crystallite size of ZnO-NPranged 50-60 nm.

Growth Performance and Survival Rate

Growth performances of juvenile *C. idella* in response to different dietary inorganic sources of Zn are shown in Table 2. No mortality was observed during the experimental period while at the end of experiment a significant difference in body weight was observed between groups of fish fed Zn supplemented diet as compared to basal diet. The highest %WG, SGR and FCE was observed in group of fish fed ZnO-NP-1 followed ZnO-NP-11 diet, while other diets supplemented with zinc in sulfated at both levels and oxide at lower level showed depressed growth (Table 2). The growth performance of fish fed ZnO-1 diet was statistically comparable to control group of fish. The HSI of fish fed ZnO-NP-1 and ZnO-NP-11 supplemented diets were statistically similar and considerably higher than that of grass carps fed other diets.

Hematological Parameters

The fish fed Zn supplemented diet showed a significant decreased in WBCs, Hb, HCT, MCV, MCH values but the increased in RBCs and MCHC values as compared to the fish fed basal diet (Table 3). Moreover, when these parameters were compared among groups of fish fed different inorganic sources of Zn supplemented diets, then all blood parameters values were higher with ZnO-NP-1 diet as compared to ZnO-NP-11, ZnO (1 and 11) and $ZnSO_4$ (1 and 11) enriched diets. Similar trend was obtained for MCHC value, considerably higher in grass carp offered ZnO-NP at lower rate (30 mg kg^{-1}) diet as compared to control and other supplemented diets (Table 3).

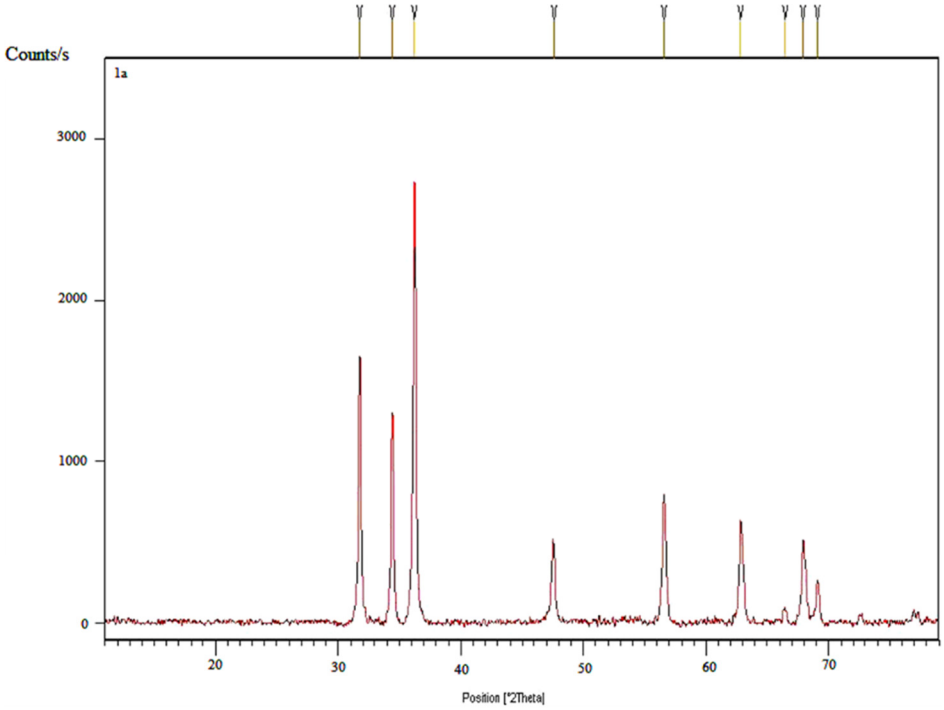

Fig. 1: XRD results of ZnO nanoparticles

Table 1: Formulation of 35% protein basal diet

Ingredients	Amount (g kg^{-1})
Fish meal	105
Soybean meal	212
Canola meal	212
Sunflower meal	212
Gluten 30%	105
Wheat bran	52
Rice polish	52
Vitamin premix[a]	20
*CMC	10
**DCP	10
Vegetable Oil	10
Total	1000

*Carboxymethyl cellulose
**Dicalcium phosphate
A (Vitamin premix contains vitamins, amino acid and minerals premix kg-1) Vitamin AB.P 40,000,000 IU, Vitamin K_3B.P 800 mg, Vitamin D_3B.P 820,000 IU, Vitamin EB.P 6200 mg, Vitamin B_2B.P 2500 mg, Vitamin B_3B.P 5100 mg, Vitamin B_{12}B.P 1000 mg, Vitamin PP B.P 10,500 mg, L. lysine B.P 10,500 mg, DL- Methionine B.P 50,500 mg, Manganese USP 30,000 mg 15,100 mg, Zinc USP 17,555 mg, Copper B.P 1000 mg, Choline chloride USP 125,500 mg, Cobalt B.P 50 mg, Iodine B.P 300 mg, Selenium B.P 80 mg

Discussion

No mortality was observed throughout the feeding trial experiment whereas significant differences in the %WG of young grass carp *C. idella* offered basal and Zn supplemented diets were observed. This revealed that rearing condition was favorable during the experiment.

The fish fed Zn in nanoparticles form showed a higher growth rate followed by oxide at lower level while depressed growth was observed in grass carps offered dietary $ZnSO_4$ supplementation at both level and ZnO at higher level (Table 2). It is well accepted that differences exist in growth rate in response to different dietary sources of Zn (Tan and Mai, 2001; Buentello *et al*., 2009) and suggested that the different chemical form of Zn showed differential bioavailability in fish. In hybrid striped bass Zn proteinate was about 1.7 more efficiently utilized than $ZnSO_4$ (Buentello *et al*., 2009). Similarly, in *Haliotis discus hannai*, dietary Zn in the Zn-Met form showed three times more bioavailability as compared to of $ZnSO_4$ (Tan and Mai, 2001). Watanabe *et al.* (1997) also reported the difference in growth rate of rainbow trout in response to same level of dietary Zn i.e., 20 mg kg^{-1} in the sulfate, nitrate and chloride forms. The growth rate was higher in response to dietary $ZnSO_4$, followed by $ZnNO_3$ and lowest was observed in fish fed $ZnCl_3$.

It is well established that Zn is vital for the growth and development of freshwater animals in certain amount but excess concentration directly through water or food chain appeared harmful and toxic (Hayat *et al*., 2007; Hao *et al*., 2013). We observed that fish fed diet supplemented with Zn in nanoform at the rate of 30 mg Zn kg^{-1} diet (Zn-NP-1) showed significantly ($P > 0.05$) higher %WG followed by Zn-NP-11 diet. This level lies within the range reported by many investigators for different fish species (Clearwater *et al*., 2002; Apines *et al*., 2001) but somewhat less than reported for hybrid striped bass (Buentello *et al*., 2009), juvenile abalone (Tan and Mai, 2001) and Atlantic salmon (Maage *et al*., 2001).

Table 2: Growth performance of Juvenile Grass carp (*Ctenopharyngodon idella*), fed basal diet and experimental diet supplemented with various forms of graded levels of Zinc

Parameter	Control	ZnO-NP-I	ZnO-NP-II	ZnO-I	ZnO-II	$ZnSO_4$-I	$ZnSO_4$-II
WBI	5.49±0.032^{a}	5.65±0.010^{a}	5.65 ± 0.07^{a}	5.55±0.027^{a}	5.73±0.04^{a}	5.54±0.08^{a}	5.76±0.06^{a}
WBF	10.27±0.03^{c}	15.33±0.12^{a}	11.80±0.11^{b}	10.49±0.05^{c}	10.45±0.1^{c}	10.1±0.18^{c}	8.97±0.01^{d}
WG	4.77±0.041^{c}	9.68±0.124^{a}	6.15±0.045^{b}	4.95±0.024^{c}	4.72±0.05^{c}	4.55±0.09^{c}	3.21±0.06^{d}
% WG	87.006±1.1^{c}	171.38±2.3^{a}	108.77±0.7^{b}	89.30±0.30^{c}	82.41±0.5^{d}	82.05±0.9^{d}	55.73±1.7^{e}
SGR	0.18±0.002^{c}	0.35±0.003^{a}	0.23±0.001^{b}	0.19±0.008^{c}	0.17±0.01^{d}	0.17±0.02^{d}	0.10±0.05^{e}
HSI %	0.53±0.001^{e}	1.50±0.015^{a}	1.46±0.017^{a}	1.22±0.024^{b}	1.20±0.02^{b}	0.94±0.01^{c}	0.83±0.04^{d}
FCR	3.95±0.003^{d}	1.94±0.005^{g}	3.06±0.001^{f}	3.81±0.002^{e}	3.99±0.001^{c}	4.14±0.001^{b}	5.87±0.01^{a}
FCE (%)	25.29±0.09^{d}	51.30±0.01^{a}	32.58±0.01^{b}	26.23±0.01^{c}	25.03±0.13^{e}	24.12 ± 0.01^{f}	17.0±0.03^{g}

Results are represented as Mean ± SE. (n=21). Means followed by different letters within the row are significantly different (P < 0.05). Control, Basal diet; ZnO, Zinc oxide; NP, nanoparticles; I, 30 mg Zinc kg^{-1} diet; II, 60 mg Zinc kg^{-1} diet; $ZnSO_4$, Zinc sulfate.

Table 3: Hematological indices of Juvenile Grass carp (*Ctenopharyngodon idella)*, fed basal diet and experimental diet supplemented with various forms of graded levels of Zinc.

Parameters	Control	ZnO-NP-I	ZnO-NP-II	ZnO-I	ZnO-II	$ZnSO_4$-I	$ZnSO_4$-II
WBC(10^3 μL^{-1})	193.46±0.49^{a}	185.6±0.05^{b}	168.53±0.17^{c}	168.36±0.08^{c}	162.26±0.08^{e}	163.73±0.08^{d}	158.56±0.08^{f}
RBC(10^6 μL^{-1})	2.84 ± 0.09^{b}	3.65 ± 0.27^{a}	2.76 ± 0.02^{b}	2.64±0.005^{bc}	2.84± 0.01^{b}	2.57±0.008^{bc}	2.05 ± 0.01^{c}
HBG (g dL^{-1})	9.23 ± 0.08^{a}	8.66±0.14^{ab}	8.5 ± 0.03^{b}	8.2 ± 0.11^{b}	8.33±0.03^{b}	8.2 ± 0.11^{b}	8.36 ± 0.08^{b}
HCT %	37.43 ± 0.14^{a}	32.36±0.08^{b}	30.66 ± 0.08^{c}	30.6 ± 0.05^{c}	24.26 ±0.34^{d}	26.93±0.08^{d}	23.7±0.11^{e}
MCV(FL)	126.13±0.14^{a}	113.43±0.12^{b}	112.76±0.08^{b}	106.53±0.18^{d}	108.73±0.63^{d}	107.26±0.14^{d}	109.7±0.05^{c}
MCH (pg)	31.46 ± 0.12^{a}	28.63 ± 0.03^{b}	26.76 ± 0.41^{c}	24.6 ± 0.05^{d}	20.66±0.08^{e}	21.3±0.11^{e}	20.23 ± 0.14^{e}
MCHC (g dL^{-1})	24.46 ±0.29^{d}	29.63 ±0.13^{a}	28.8 ± 0.05^{a}	27.5 ± 0.28^{b}	25.8± 0.05^{c}	27.2 ± 0.05^{b}	27.5±0.11^{b}

Results are represented as Mean ± SE. (n=21). Means followed by different letters within the row are significantly different (P < 0.05). Control, Basal diet; ZnO, Zinc oxide; NP, nanoparticles; I, 30 mg Zinc kg^{-1} diet; II, 60 mg Zinc kg^{-1} diet; $ZnSO_4$, Zinc sulfate

The shape, size, optical and electrical characteristics of materials are related to its physiochemical properties of material whereas reduction of macromolecule to nanoscale, alters these properties and increased their application (Alishahi *et al*., 2011; Rather *et al*., 2011). In this study a significantly (P < 0.05) higher %WG, SGR and improved FCR of fish fed ZnO-NP supplemented diet compared to $ZnSO_4$ and ZnO enriched diet at the same level may be due to small size (50-60 nm) of nanoform of ZnO. It is well know that the nanoform of particles have higher intestinal absorption, bioavailability and catalytic activities (Albrecht *et al*., 2006; Dube *et al*., 2010; Alishahi *et al*., 2011). Therefore, it might possible that conversion of ZnO in nanoform increase the efficiency of Zn by enhancing its absorption and bioavailability in the gastrointestinal tract. A higher HSI value of fish fed ZnO-NP-1 and ZnO-NP-11 diets indicate the higher energy reserve due to higher availability of nutrients and also support the view that nanoparticle form of ZnO has higher efficiency compared to other inorganic form of Zn.

Many investigators have suggested the role of Zn in the growth, development and physiology of animals (Eide, 2006; Maret and Krężel, 2007) and suggested its role in the synthesis of growth hormone (Imamoğlu *et al*., 2005). Therefore, positive effect of Zn nanoparticles on growth performance may be attributed to somatic growth by stimulation of DNA and RNA synthesis and cell division (Siklar *et al*., 2003).

It is well known that deficiency of Zn leads to growth retardation (Lim *et al*., 1996) and immunological impairment in fish (Kiron *et al*., 1993). Moreover higher intake of Zn cause deleterious effects on fish growth (Hayat *et al*., 2007). In our feeding experiment, all inorganic form of Zn at lower level (30 mg kg^{-1} diet) except nanoform enriched diet showed no profound effect on the growth performance of Juvenile Grass Carp while depressed growth was observed when fish were fed with $ZnSO_4$ or ZnO at higher concentration i.e., 60 mg kg^{-1} diet. It may be due to negative effect of Zn on the transport and absorption of other metals like iron, copper and cadmium (Heijerick *et al*., 2002). Although Zn toxicity is rare but it is advisable to avoid high Zn supplementation because it stops the loading of other minerals necessary for normal growth and physiology of fish (Buentello *et al*., 2009).

Hematological parameters are very helpful in judgment of health conditions of fish and now commonly used as an effective index for monitoring the physiological and pathological changes in fish (Kori-Siakpere *et al*., 2008). In our study we have tried to investigate the effect of graded level of different inorganic form of dietary Zn on the blood parameters. The erythrocyte indices like MCV, MCH and MCHC values showed a broad range of physiological variation in response to different inorganic form of Zn supplemented diet. Fish fed Zn supplemented diet showed a significant decrease in Hb, HCT, MCV, MCH values but increased in MCHC value as compared to the fish fed control diet (Table 3). Kori-Siakpere *et al*. (2008) also observed the significant dose dependent decreased in RBCs count, hemoglobin and hematocrit values in response to sub lethal concentration of Zn and reported that these factors affect the other hematological indices like MCV, MCH and MCHC values and collectively caused anemic condition in *Heteroclarias* sp. Although, common carp and rainbow trout can tolerate 1700 to 1900 mg Zn per kg diet without

showing any adverse effect on growth and survival of fish (Jeng and Sun, 1981; Wekell *et al*., 1983) but elevated level i.e., 1000 mg kg^{-1} diet caused decrease in Hb level and HCT value in rainbow trout (Knox *et al*., 1984).

In the present study, the difference in hematological variables related to oxygen transport (RBCs, Hb, HCT) and calculated indices (MCV, MCH and MCHC) in response to basal and Zn supplemented diet possibly be related to hemodilution or hemoconcentration due to alteration in blood water content (Oti and Avoaja, 2005). A decrease in HCT value of grass carp in response to all Zn supplemented diets in the present study indicated hemodilution in response to Zn (Oti and Avoaja, 2005; Kori-Siakpere *et al*., 2008; Olurin *et al*., 2012; Celik *et al*., 2013).

The dose dependent reduction in RBCs of grass carp fed $ZnSO_4$ and ZnO supplemented diet may be due to the swelling of the red cells that lead to hemolysis. Hemolysis of erythrocytes has also been reported in *Heteroclarias* (Oti and Avoaja, 2005; Kori-Siakpere *et al*., 2008) and rainbow trout (Koyama *et al*., 1982) in response to Zn. Similarly, according to Kori-Siakpere *et al*. (2008), the reduction in erythrocyte indices is related to of anemic condition.

A comparison of blood parameters of fish fed different form of dietary inorganic source of Zn revealed that all blood parameters were significantly higher for fish fed diet containing ZnO-NP at lower rate (30 mg kg^{-1}) as compared to other Zn supplemented diets. It seems that Zn in nanoform was more efficiently absorbed, utilized and showed no negative impact on the absorption and bioavailability of other trace elements (Buentello *et al*., 2009). Moreover, significantly higher RBCs count and MCHC value in response to Zn-NP-1 diet as compared to control or other Zn supplemented diets may be due to an increase in blood cell reserve combine with cell shrinkage due to Zn-induced the osmotic alteration of blood (Tort and Torres, 1988).

In all vertebrates including fish, the WBCs count increase or decrease in response to various stressors like infections and chemical pollutant (Olurin *et al*., 2012; Moharram *et al*., 2011). We observed significant decrease in WBCs count in all group of fish fed Zn supplemented diet as compared to basal diet. Like our results, other scientists also reported the decrease in WBCs count in *Clarias* and "*Heteroclarias*" species (Oti and Avoaja, 2005; Kori-Siakpere *et al*., 2008) in response to Zn. The decreased in number of white blood cells (leucopaenia) in the present study or previous studies may either be the result of bioaccumulation of Zn in different tissues that cause toxicity and effect on cell production from spleen (Firat, 2007) or due to an increased level of corticosteroid hormones (Celik *et al*., 2013) because these hormones are important for prevention and healing of inflammation.

Conclusion

The level of Zn in sulfated and oxide form used in the present study were somewhat higher than required for this species, *C. idella* and better results can be achieved by lowering the levels. Furthermore, profound effect of nanoform of Zn at lower level on both growth performance and RBCs count suggests that it improved the efficiency due to higher absorption, bioavailability and non-toxic effect on the absorption of other trace metals. Moreover, results of present study indicate the scope of nanotechnology for the enhancement of fish production.

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