Dietary zinc requirement of Labeo rohita juveniles fed practical diets

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ABSTRACT
The present study was designed to estimate the zinc (Zn) requirement of Labeo rohita juveniles fed practical diet. Treatments used for the study were consisted of six experimental diets supplemented with graded levels of Zn (0, 21, 42, 63, 84 and 104 mg/kg diet) from Zn gluconate. For each experimental diet, two replicates were allocated, and 18 fish were stocked in each replicate. The feeding trial was lasted for 90 days. Results showed that final weight, absolute weight gain, weight gain% and specific growth rate increased with increasing dietary Zn levels up to 42 mg/kg and started to decrease with further increase in dietary Zn level. Quadratic regression analysis of weight gain% data indicated that L. rohita juveniles required 62.58 mg/kg Zn for normal growth. Maximum Zn absorption was observed in fish fed diet supplemented with 42 mg/kg Zn compared with other dietary treatments. Alkaline Phosphatase (ALP) activity in kidney and spleen of L. rohita juveniles increased with the increase in dietary Zn levels up to 42 and 63 mg/kg, respectively. Conclusively, supplementation of graded levels of dietary Zn-gluconate improved the growth performance and increased the Zn bioavailability and ALP activity up to a certain limit in L. rohita juveniles.

Introduction
Mineral elements are vital nutrients as they play an important role in maintaining osmoregulation and formation of bones and scales. Trace minerals are mainly absorbed from water which make it difficult to determine their requirements for fish (National Research Council 1983).

Zinc (Zn) is an important inorganic trace element as it influences various physiological functions including growth performance, immune system and defense mechanism against free ions and radicals (Watanabe et al. 1997). As a cofactor, Zn participates in several enzyme systems to regulate protein and lipid metabolism (National Research Council 2011). It plays a key role in the formation of nucleoproteins as well as in prostaglandin metabolism (Watanabe et al. 1997). Korenek et al. (2007) observed as increase in the activities of chymotrypsin and trypsin in the droppings of quails by the supplementation of Zn. Alexei et al. (2002) reported that Zn stabilizes the activities of amylase and lipase in bacteria.

Growing aquatic species do not obtain the enough Zn because freshwater (Spry et al. 1988) and seawater (Willis and Sunda 1984) are deficient in normal Zn levels. Hence, Zn is considered an essential trace mineral in fish feeds (Lall 1989; National Research Council 1993; Wei et al. 1999). Zn appears to be less bioavailable to the fish fed high plant meal based diets as such diets contain low level of Zn and high levels of phytate, an anti-nutritional factor. Presence of phytate adversely affected the bioavailability of Zn in chinook salmon (Richardson et al. 1985). Similar results were also observed in channel catfish (Gatlin and Wilson 1984; Gatlin and Phiflip 1989). The requirement of dietary zinc for the juvenile grouper ranged between 28.9 and 33.7 mg/kg diet (Houng-Yung et al. 2014). Whereas, optimal level of total dietary Zn in practical diet (supplemented and contributed by ingredients) of rainbow trout was found to be 80 mg kg (Welker et al. 2016).

Zn deficiency caused reduced growth rate, increased mortality, low body weight, skeletal deformities, cataracts and fin and skin erosion in fish (Tacon 1992). Nucleic acid and protein metabolism (Lall 2002) disorders were also observed in salmonids due to reduced activity of Zn-requiring enzymes (Ramseyer et al. 1999; Kucukbay et al. 2006; Rider et al. 2010). Therefore, adequate amount of Zn is required to avoid such deficiency symptoms. Zn sulphate (ZnSO4. 7H2O), an inorganic source, is usually used as Zn source to determine the requirements in fishes.

Recently, organically chelated trace elements are being supplemented in aquafeeds instead of inorganic minerals. Organic minerals are structurally stable and have low molecular weight, so, they compete with mineral inhibitors and increases the uptake of mineral in fish intestine (Ashmead 1992, 1993). Therefore, it may hypothesize that organic minerals are more bioavailable to the fish compared to inorganic salts (Zn-sulphate and Zn-oxide) (Ashmead 1992). Utilization of organic minerals in feeds is like natural process of micro-mineral supplementation as natural feed ingredients consist of mineralized proteins and amino acids (Tucker and Taylor-Pickard 2005).

Indian major carps (Labeo rohita, Catla catla, and Cirrhinus mrigala) shares significant contribution in freshwater production.
of Indian subcontinent. The demand of *L. rohita* is dominated in the market due to its unique flavour, rapid growth and disease resistance capacity (Jhingran and Pullin 1988). However, dietary Zn requirement has not been determined until now for *L. rohita* juveniles. Therefore, the purpose of the present study is to evaluate the Zn requirement from Zn-gluconate, an organic source, in practical diet for *L. rohita* juveniles.

**Materials and methods**

**Feed ingredients and experimental diets**

The present research work was carried out to study the Zn requirement from Zn gluconate, an organic source, in practical diet of *L. rohita* juveniles. By supplementing Zn at the levels of 0, 21, 42, 63, 84 and 104 mg/kg diet, six isolipidic, isocaloric and isonitrogenous experimental diets were formulated from the basal diet (Table 1). Feed ingredients were purchased from a commercial feed mill and experimental diets were formulated using these ingredients which were analyzed chemically (AOAC 1995). Cereal grinding machine (FFC-45, JIMO, China) was used to grind and sieve (0.05 mm) the feed ingredients. By using an electric mixer, chromic oxide, was used to grind and sieve (0.05 mm) the feed ingredients. Composition of Zn free mineral mixture and vitamin premix were blended thoroughly. Composition of Zn free mineral mixture is presented in Table 2. The dough was prepared by adding 150 ml of distilled water/kg of diet and pelleted with hand pelletizer having a diameter of 3 mm. The moist pellets were dried at room temperature till the moisture was reduced up to 10% using an electric fan. Pellets were broken in small pieces, screened to desired sizes and refrigerated at −20°C in self-sealing plastic bags until fed. The proximate composition of experimental diets is given in Table 3.

| Table 1. Composition of basal practical diet. |
|---------------------------------------------|
| Ingredient                     | Percentage (%) |
| Soybean meal                      | 30              |
| Corn-gluten                      | 23              |
| Sunflower meal                    | 20              |
| Fishmeal                         | 15              |
| Fish oil                         | 5               |
| Wheat flour                       | 4.5             |
| Vitamin premixa                  | 1               |
| Zn free mineral mixture          | 0.5             |
| Total                           | 100             |

Notes: Vitamin A 15 M.I.U. Vitamin D3 3 M.I.U. Nicotinic acid 25,000 mg; Vitamin B1 5000 mg Vitamin E 6000 IU Vitamin B2 6000 mg; Vitamin K3 4000 mg Vitamin B6 4000 mg Folic acid 750 mg; Vitamin B12 9000 mg Vitamin C 15,000 mg Calcium pantothenate 10,000 mg.

*Each Kg of vitamin premix contains.*

| Table 2. Composition of Zn free mineral mixture. |
|-----------------------------------------------|
| Salt                                           |
| mg/g                                          |
| CaCO3                                         | 316             |
| KH2PO4                                        | 479             |
| MgSO4·7H2O                                    | 153             |
| NaCl                                          | 51              |
| CoCl2·6H2O                                    | 0.0816          |
| AlCl3·6H2O                                    | 0.255           |
| CuSO4·5H2O                                    | 210.67          |
| FeSO4·7H2O                                    | 100.67          |
| MnSO4·5H2O                                    | 1116.67         |

**Fish husbandry**

*L. rohita* juveniles were obtained from Government Fish Seed Hatchery. At arrival, juveniles were dipped in 5 g/L NaCl solution to reduce the incidence of ectoparasites and fungal infection. Fish were acclimatized in cemented tanks (1000 L water capacity) for two weeks. Juveniles were fed with basal diet to apparent satiation level. Eighteen juveniles with an average initial weight of 3.15 ± 0.01 g were assigned to each V-shaped tank (70 L capacity) for the feeding trial. The *L. rohita* juveniles were hand-fed once per day to the apparent satiation level for 3 months. After feeding session of three hours, the valves were opened to clean the tanks thoroughly and uneaten diet was drained out from the tanks by manual siphoning and filtered fresh water was supplied to each tank. The oxygen level was maintained in the rearing tanks through the capillary system.

**Growth performance**

Growth in term of absolute weight gain, weight gain%, feed conversion ratio (FCR) and specific growth rate (SGR) was calculated by standards formulae.

\[
\text{Absolute Weight gain (g)} = \text{Final weight gain (g)} - \text{Initial weight gain (g)}
\]

\[
\text{Weight gain%} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100
\]

\[
\text{FCR} = \frac{\text{Total dry feed intake (g)}}{\text{Wet weight gain (g)}}
\]

\[
\text{SGR} = \frac{\ln \text{Initial weight (g)} - \ln \text{Final weight (g)}}{\text{No. of days}} \times 100
\]

**Sample collection**

Fish were starved for 24 hours and sacrificed using anaesthesia (MS-222) at the end of the feeding trial. Ten fish were selected to obtain muscles, kidney, intestine, bones, scales and spleen samples. Each pooled sample was reffergerated at −20°C until used for the determination of alkaline phosphatase activity (ALP), thiobarbituric acid reactive substances (TBARS), proximate composition and Zn content analysis.

**Proximate analysis**

The standard methods of AOAC (1995) were followed to determine the proximate composition of experimental diets and muscle samples. Moisture was determined by drying the samples at 105°C for 12 h. Crude protein was measured by using Kjeldahl apparatus and petroleum aether extraction method was used to determine crude fat. Crude ash contents were obtained by igniting the samples at 600°C for 12 h in muffle furnace.

**Zn content analysis**

For this purpose, nitric acid and perchloric acid were used in 3:1 ratio (Wet digestion) to digest the samples of (bones, scales and
Table 3. Proximate composition of experimental diets.

| Experimental Diets | Zn-gluconate Level (mg/kg) | Dry matter (%) | Crude protein (%) | Crude fat (%) | Crude ash (%) | Gross energy (kcal/kg) | Zn (µg/g) |
|--------------------|---------------------------|----------------|------------------|--------------|--------------|------------------------|-----------|
| Zn1                | 0                         | 90.82          | 34.92            | 7.08         | 8.16         | 3926.875               | 43.97     |
| Zn2                | 21                        | 90.4           | 35.34            | 7.04         | 8.6          | 4000.905               | 60.44     |
| Zn3                | 42                        | 90.825         | 34.89            | 7.46         | 8.785        | 4074.865               | 80.39     |
| Zn4                | 63                        | 91.005         | 35.549           | 7.35         | 9.145        | 4002.54                | 101.03    |
| Zn5                | 84                        | 90.895         | 35.644           | 7.045        | 9.495        | 3958.665               | 117.13    |
| Zn6                | 104                       | 91.125         | 35.0045          | 6.93         | 9.7          | 4009.015               | 140.99    |

intestine) digested samples were diluted to the appropriate level. Atomic absorption spectrophotometer was used to determine the Zn contents in samples (AOAC 1995).

Zn absorption

After three hours of feeding all uneaten diet was collected for determination of FCR and tanks were cleaned with filtered fresh water. Again, after two hours of tank cleaning, the faecal material was collected with care to avoid thin breakage of thin faecal strings through faecal collection tube of V-shaped tanks equipped with two valves. Collected faecal material was dried at 60°C, minced and refrigerated for the analysis of Zn contents. Absorption of Zn was estimated by the following formula:

\[
\text{Absorption} \% = 100 - 100 \times \left( \frac{\text{Percent marker in diet} \times \text{Percent nutrient in feces}}{\text{Percent marker in feces} \times \text{Percent nutrient in diet}} \right)
\]

TBARS assay

For the estimation of peroxidation level in kidney and spleen, TBARS contents were measured by following Gatta et al. (2000).

ALP enzyme assay

ALP activity in kidney and spleen of L. rohita juveniles was determined using Vitro Scient (ISO 13485) kit method. The ALP of the sample catalyses the magnesium-activated base hydrolysis of p-nitro-phenyl phosphate producing nitrophenolate and absorbance was measured at 405 nm. The activity of enzyme was directly proportional to the rate of hydrolysis (Abbott Laboratories 1989).

Statistical analysis

The data were tested for normality and homogeneity of variances and finally, one-way analysis of variance was used for statistical analysis of data (Steel et al. 1996). The differences among different supplementation levels of Zn were compared by Tukey’s Honestly significant difference test (Snedecor and Conrhan 1991). Quadratic regression was applied for the determination of optimum requirement of Zn. All of the statistical analysis was performed using CoStat computer package (Version 6.303, PMB 320, Monterey, CA, 93940 USA).

Results

Effect of graded levels of Zn on growth performance of L. rohita juveniles is given in Table 4. Final weight, absolute weight gain, weight gain% and SGR were increased with increase in dietary Zn levels up to 42 mg/kg and decreased with further increase in dietary Zn level, whereas, feed conversion ratio was reduced at this level (42 mg/kg) in L. rohita juveniles. Survival rate remained unaffected with the increase in Zn gluconate levels.

The data of weight gain% was analysed by the quadratic regression analysis to determine the requirement of Zn in diet for L. rohita juveniles. The result indicated that L. rohita required 62.58 mg/kg Zn for optimum growth (Figure 1).

Effect of graded levels of Zn on muscle proximate composition of L. rohita juveniles is summarized in Table 5. Non-significant variations were observed among all the dietary treatments for dry matter, crude protein, crude fat and crude ash contents in muscles of L. rohita juveniles.

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Table 4. Effect of graded levels of Zn on growth performance of L. rohita juveniles.

| Zn-gluconate levels (mg/kg) | Experimental diets | 0 | 21 | 42 | 63 | 84 | 104 | p-value |
|-----------------------------|--------------------|---|----|----|----|----|-----|--------|
| Final weight (g)            | Zn1                | 15.03 ± 0.12² | 16.1 ± 0.601¹ | 17.57 ± 0.05³ | 17.37 ± 0.07² | 16.92 ± 0.12² | 16.44 ± 0.16³ | <0.0001*** |
| Absolute weight gain (g)    | Zn2                | 11.89 ± 0.07³ | 12.96 ± 0.068¹ | 14.39 ± 0.06³ | 14.22 ± 0.09³ | 13.78 ± 0.10³ | 13.29 ± 0.15³ | <0.0001*** |
| Weight gain (%)             | Zn3                | 378.83 ± 2.64³ | 412.1 ± 20.26³ | 453.38 ± 3.01³ | 451.59 ± 4.94³ | 437.61 ± 3.01³ | 421.89 ± 3.04³ | <0.0001*** |
| Specific growth rate (%)    | Zn4                | 1.74 ± 0.006³ | 1.81 ± 0.04³ | 1.90 ± 0.06³ | 1.89 ± 0.09³ | 1.86 ± 0.00³ | 1.83 ± 0.00³ | <0.0001*** |
| FI (g)                      | Zn5                | 18.92 ± 0.24³ | 16.97 ± 0.106³ | 16.52 ± 0.03³ | 16.85 ± 0.07³ | 17.15 ± 0.07³ | 17.12 ± 0.10³ | <0.0001*** |
| FCR                         | Zn6                | 1.59 ± 0.01³ | 1.31 ± 0.06³ | 1.14 ± 0.00³ | 1.18 ± 0.003³ | 1.24 ± 0.01³ | 1.28 ± 0.00³ | <0.0001*** |
| Survival rate (%)           |                   | 97.22 ± 3.92² | 100 ± 0 94.44 ± 0 | 94.44 ± 7.85 | 100 ± 0 97.22 ± 3.92 | 5876 ns |        |

Notes: The data are means of two replicates. The data are presented in means ± standard deviation. Mean values sharing different superscript letters within a row are significantly different (p < 0.05). FI = feed intake; FCR = feed conversion ratio.
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Figure 1. Graphical representation showing the dietary requirement of Zn for \(L. \text{rohita}\) juveniles.

Discussion

Among trace minerals, Zn is an important nutrient as it is involved in various metabolic pathways such as protein synthesis, growth, immunity and energy metabolism in fish like other animals (Hayashi et al. 2001; Carpene et al. 2003; Lin et al. 2013; Houng-Yung et al. 2014). It performs its functions as a cofactor in more than 200 metalloenzymes including DNA polymerase, carbonic anhydrase and carboxy peptidase (Salim et al. 2012). The importance of Zn as antioxidant has also been illustrated in many aquatic organisms (Feng et al. 2011; Trevisan et al. 2014; Huang et al. 2015). However, high Zn ingestion showed negative effects on feed utilization and growth in Jian carp, \(Cypinus \text{carpio} \) var. Jian (Tan et al. 2011), yellow catfish, \(Peltobagrus \text{fulvidraco}\) (Luo et al. 2011) and Nile tilapia, \(Oreochromis \text{niloticus}\) (Do Carmo e Sa et al. 2004). Therefore, it is necessary to supplement the optimum level of dietary Zn to avoid negative effects on fish health. Its optimum supplementation may reduce the feed cost and mineral leaching in aquatic water bodies (Buentello et al. 2009; Huang et al. 2015).

In the current study, final weight, absolute weight gain, weight gain% and SGR increased with increasing dietary Zn gluconate levels up to 42 mg/kg and decreased with further increase in dietary Zn level whereas FCR was reduced at this level (42 mg/kg) in \(L. \text{rohita}\) juveniles. Survival rate remained unaffected at all levels of Zn. Like our results, a significant increment was also observed in SGR of juvenile Jian carp fed Zn supplemented diet (Tan et al. 2011). Similar observations were also recorded by Gatlin and Wilson (1983), Do Carmo e Sa et al. (2004) and Ogino and Yang (1979) in channel catfish, Nile tilapia and common carp, respectively. Moreover, weight gain of juvenile grouper was significantly increased by increasing the concentration of Zn in diet up to 36.7 mg/kg and then levelled off with further increase in dietary Zn level, which may owe to the pro-oxidative effect of Zn (Houng-Yung et al. 2014). Bhagawati et al. (2016) reported improved weight gain in golden mahseer fry fed diet supplemented with 40 mg/kg Zn and decreased significantly with further increase in dietary Zn level. Similarly, findings of Liang et al. (2012) and Tan et al. (2011) are also in accordance with our results that higher

Table 5. Effect of graded levels of Zn on muscle proximate composition of \(L. \text{rohita}\) juveniles.

| Experimental diets | Zn-gluconate levels (mg/kg) | 0    | 21   | 42   | 63   | 84   | 104  | p-value |
|--------------------|-----------------------------|------|------|------|------|------|------|---------|
| Dry matter (g/kg)  | Zn1                         | 239.85 ± 0.54 | 242.03 ± 0.92 | 239.7 ± 0.93 | 239.96 ± 2.10 | 241.57 ± 1.49 | 239.51 ± 0.80 | .3201 ns |
|                    | Zn2                         | 237.7 ± 0.35 | 240.5 ± 1.58 | 237.2 ± 1.83 | 237.36 ± 3.12 | 238.97 ± 2.51 | 237.8 ± 0.91  | .2839 ns |
|                    | Zn3                         | 235.6 ± 0.24 | 238.4 ± 1.48 | 234.1 ± 1.75 | 234.34 ± 3.08 | 235.95 ± 2.41 | 234.8 ± 0.85  | .9346 ns  |
|                    | Zn4                         | 233.5 ± 0.13 | 236.3 ± 1.30 | 232.8 ± 1.57 | 233.14 ± 2.95 | 234.75 ± 2.36 | 233.6 ± 0.81  | .9608 ns  |
|                    | Zn5                         | 231.4 ± 0.04 | 234.2 ± 1.10 | 230.7 ± 1.37 | 231.04 ± 2.85 | 232.65 ± 2.26 | 231.5 ± 0.76  | .9808 ns  |
|                    | Zn6                         | 229.3 ± 0.01 | 232.1 ± 0.96 | 228.6 ± 1.23 | 228.94 ± 2.65 | 229.55 ± 2.16 | 228.4 ± 0.72  | .9808 ns  |

Notes: The data are means of two replicates. The data are presented in means ± standard deviation. Mean values sharing different superscript letters within a row are significantly different (p < .05).

Table 6. Effect of graded levels of Zn on Zn contents (µg/g) in bones, scales, intestine and faeces of \(L. \text{rohita}\) juveniles.

| Experimental diets | Zn-gluconate levels (mg/kg) | 0    | 21   | 42   | 63   | 84   | 104  | p-value |
|--------------------|-----------------------------|------|------|------|------|------|------|---------|
| Bones              | Zn1                         | 136.5 ± 0.33d | 138.58 ± 0.34c | 141.57 ± 1.16b | 146.00 ± 0.13a | 147.5 ± 0.38b | 146.0 ± 0.23a | .0000***  |
|                    | Zn2                         | 90.69 ± 0.01b | 94.95 ± 0.03c | 94.99 ± 0.04b | 94.9 ± 0.05a  | 92.84 ± 0.03b | 90.69 ± 0.07c | .0000***  |
|                    | Zn3                         | 357.07 ± 0.18d | 357.07 ± 0.18d | 357.07 ± 0.18d | 357.07 ± 0.18d | 357.07 ± 0.18d | 357.07 ± 0.18d | .0000***  |
|                    | Zn4                         | 60.3 ± 0.13b | 60.3 ± 0.13b | 60.3 ± 0.13b | 60.3 ± 0.13b | 60.3 ± 0.13b | 60.3 ± 0.13b | .0000***  |
|                    | Zn5                         | 86.22 ± 2.11e | 86.22 ± 2.11e | 86.22 ± 2.11e | 86.22 ± 2.11e | 86.22 ± 2.11e | 86.22 ± 2.11e | .0000***  |
|                    | Zn6                         | 102.65 ± 1.15d | 102.65 ± 1.15d | 102.65 ± 1.15d | 102.65 ± 1.15d | 102.65 ± 1.15d | 102.65 ± 1.15d | .0000***  |

Notes: The data are means of two replicates. The data are presented in means ± standard deviation. Mean values sharing different superscript letters within a row are significantly different (p < .05).

Table 7. Effect of graded levels of Zn on TBARS (mg/g protein) contents in kidney and spleen of \(L. \text{rohita}\) juveniles.

| Experimental diets | Zn-gluconate levels (mg/kg) | 0    | 21   | 42   | 63   | 84   | 104  | p-value |
|--------------------|-----------------------------|------|------|------|------|------|------|---------|
| Kidney             | Zn1                         | 1.90 ± 0.04a | 1.63 ± 0.04b | 1.36 ± 0.04c | 1.06 ± 0.04d | 1.02 ± 0.04e | 1.43 ± 0.04f | .0000***  |
|                    | Zn2                         | 1.5 ± 0.14c | 1.09 ± 0.14d | 0.90 ± 0.04c | 0.76 ± 0.04b | 1.46 ± 0.09b | 1.5 ± 0.04a  | .0005***  |
| Spleen             | Zn1                         | 1.90 ± 0.04a | 1.63 ± 0.04b | 1.36 ± 0.04c | 1.06 ± 0.04d | 1.02 ± 0.04e | 1.43 ± 0.04f | .0000***  |
|                    | Zn2                         | 1.5 ± 0.14c | 1.09 ± 0.14d | 0.90 ± 0.04c | 0.76 ± 0.04b | 1.46 ± 0.09b | 1.5 ± 0.04a  | .0005***  |

Notes: The data are means of two replicates. The data are presented in means ± standard deviation. Mean values sharing different superscript letters within a row are significantly different (p < .05). TBARS = Thiobarbituric acid reactive substances.
dietary Zn levels increased the FCR in Ctenopharyngodon idella and Cyprinus carpio to a certain limit. In contrast to our results, weight gain in Nile tilapia remained unaffected when fed 150 mg/kg dietary Zn as Zn oxide, Zn amino acid complex or Zn sulphate monohydrate (Do Carmo e Sa et al. 2005). Luo et al. (2011) observed no significant effect of Zn supplementation on feed intake in yellow catfish.

In the current study, non-significant changes were observed in dry matter, crude protein, crude fat and ash contents of L. rohita juveniles fed on Zn-glucosone supplemented diets. Similar to our study, crude protein and moisture contents in the whole body remained unaffected among different dietary Zn treatments in turbot (Ma et al. 2014). Contrast to our results, enhanced body lipid content and decreased moisture and ash contents were observed when fish were fed higher Zn level (53 mg/kg) (Liang et al. 2012). Ma et al. (2014) reported improved ash and crude lipid contents in turbot fed Zn supplemented diets. This may be due to increase in anti-oxidative activities (superoxide dismutase and glutathione peroxidase) that significantly lower the lipid peroxidation thereby increasing the lipid contents in turbot (Mallick and Mohn 2000).

Trace minerals as essential nutrients are vital to maintain the structural and functional activities in fish as well as in terrestrial animals. Bone mineral contents are always considered a sensitive indicator for estimating the mineral status of an organism. In the present study, Zn absorption increased up to 42 mg/kg dietary Zn level and then decreased with further increase in dietary Zn level. Similar effects were recorded in various tissues of rats, rainbow trout and channel catfish by Huber and Gershoff (1973), Apines et al. (2001) and Gatlin and Wilson (1983). Alkaline phosphatase (ALP) activity is also considered a sensitive indicator of Zn status in animal (Swinkels et al. 1996). In the present study, supplementation of dietary Zn showed dose dependent effect on ALP activity in kidney and spleen. Increased ALP activity may attribute to the increased Zn availability to the fish as Zn acts as cofactor for this enzyme. Dietary Zn concentration strongly influenced the activity of ALP in juvenile abalone (Tan and Mai 2001). The study conducted by Do Carmo e Sa et al. (2004) on Nile tilapia showed a significant increase in ALP activity in response to dietary Zn supplementation up to 50 mg/kg Zn level. Similar effects were recorded in various tissues of rats, rainbow trout and channel catfish by Huber and Gershoff (1973), Apines et al. (2001) and Gatlin and Wilson (1983).

Absorption of Zn occurs mainly in upper small intestine and usually follows the first order kinetics (Wood et al. 2011). In the current study, Zn absorption increased up to 42 mg/kg dietary Zn level and then decreased with further increase in dietary Zn level. Similar to our study, amino acid chelated Zn (an organic source) showed higher absorption of Zn in rainbow trout at 40 mg/kg Zn level (Apines et al. 2001).

Conclusively, supplementation of graded levels of dietary Zn-glucosone improved the growth performance and Zn bioavailability up to a certain level while higher showed negative influence on the performance of L. rohita juveniles.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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