

Effect of Zinc Sulfate on Growth, Immune Response and Antioxidative of *Misgurnus anguillicaudatus*

Bi Hui and Wen An-Xiang
College of Biology and Science, Sichuan Agricultural University,
625014 Ya'an, Sichuan, China

Abstract: This study was designed to determine the dietary zinc requirement of *Misgurnus anguillicaudatus* by decision the effect of the graded levels of dietary zinc on growth, immune response and antioxidative. A total of 540 *Misgurnus anguillicaudatus* (initial weight 3.3 ± 0.1 g) were randomly distributed into 6 groups of each three replicates, feeding diets containing graded levels of zinc (0, 15, 30, 60, 90 and 120 mg kg⁻¹ diet) for 80 days each group were sampled to measure weight gain, Specific Growth Rate (SGR), relative fatness and feed efficiency; protease, lipase and amylase activities in intestine and hepatopancreas the hago-rate erythrocyte, survival rate after vaccination, the Alkaline Phosphatase (AKP) and lysozyme activity in plasma; the Total Antioxidant enzymes (T-AOC), Catalase (CAT), Superoxide Dismutase (SOD), CuZn superoxide dismutase (CuZn-SOD) activities and Malondialdehyde (MDA) in plasma and hepatosomatic. Results indicate that the weight gain rate and SGR of dietary treatments were significantly the highest than group A. The group C was great significantly increased trypsin, lipase and amylase activities in intestine and hepatopancre. Zinc great significantly increased the hago-rate erythrocyte. AKP and lysozyme activity of group C in plasma were the highest than group A. The activities of T-AOC, CAT, SOD and CuZn-SOD of group C in plasma and hepatosomatic were great significantly increased than group A. The MDA of group C in plasma and hepatosomatic was great significantly decreased than group A. In summary, the optimal dietary zinc content for optimum growth performan, digestive ability and immune function in *Misgurnus anguillicaudatus* was about 30 mg kg⁻¹ of dietary zinc.

Key words: *Misgurnus anguillicaudatus*, zinc, digestive enzyme activities, non-specific immunity, antioxidative

INTRODUCTION

Misgurnus anguillicaudatus an Cypriniformes, Misgurnus (Ruihua, 1994) is considered as delicious food with high level of nutrition value and high medical function (Chuanguang *et al.*, 2002). It is anticipated that an expanded and consistent requirement of loach production will ultimately require the development of all kinds of culture systems. There are some negative effects of culture systems as juvenile fish death rate is higher, a large number of antibiotics and chemicals is outstanding, drug residues and so on. Therefore, the aim of this study was to dietary zinc supplementation improves *Misgurnus anguillicaudatus* growth performance and promoted the development and function of the intestine.

Zinc is an essential element for all forms of life. It is one of many factors maintain normal physiological activity of the enzyme (Wangbao *et al.*, 2007; Pang and Zhang, 2002). In fact, zinc deficiency has been associated with fish growth and immune response disturbance

(Eid and Ghonimb, 1994). Zinc supplementation shows that it can improve growth performance, immune response and antioxidant capacity of animals (Reeves *et al.*, 2001; Powell, 2000). However, no studies have been performed to data to identify whether the zinc could have beneficial effects *Misgurnus anguillicaudatus*. The aim of this study was to dietary ZnSO₄ · 7H₂O supplementation improves *Misgurnus anguillicaudatus* growth performance and promoted non-specific immunity and antioxidative function of the intestine.

MATERIALS AND METHODS

Animal and diet preparation: *Misgurnus anguillicaudatus* which is from the Chengdu fisheries were used in this experiment. Before starting the experiment, the fish was disinfected 10 min with 1.5% NaCl and were assigned to each of 18 experimental aquaria (60 L×45 W×45 H cm) connected to a closed water and oxygen auto-supplemented system. The fish were

Table 1: Composition and nutrient levels of basal diet %

| Ingredients | Contents (%) | Items | Nutrient level |
|----------------|--------------|----------------------------|----------------|
| Fish meal | 20.0 | CP (%) | 33.74 |
| Soybean meal | 35.2 | CF (%) | 5.47 |
| Corn | 17.0 | Crude ash (%) | 6.09 |
| Flour | 10.0 | P (%) | 1.35 |
| Wheat bran | 6.0 | Ca (%) | 1.54 |
| Rapeseed meal | 4.0 | Cu (mg kg ⁻¹)* | 2.75 |
| Premix | 3.0 | Zn (mg kg ⁻¹)* | 27.76 |
| Soybean oil | 2.0 | - | - |
| MCP | 1.5 | - | - |
| Binder (CMC) | 1.0 | - | - |
| Sodiumchloride | 0.3 | - | - |

The other nutrients in the table are values measured. Fish with premix adding amount of 3% (By Mu Zhi Lin feed factory provides), every 1 kg feeds including: VD 3800 IU, VE 50 IU, VA 4000 IU, VB₁ 2.5 mg, VB₂ 9 mg, VB 610 mg, VC 250 mg, nicotinic acid 40 mg, calcitriol 30 mg, VH 100 µg, choline 1000 mg, Fe 140 mg, Cu 2.5 mg, Mn 19 mg, Mg 230 mg, Co 0.1 mg, I 0.25 mg, Se 0.2 mg

acclimated to the experimental conditions for 15 days. During acclimation, fish were fed to satiate on two times a day. Formulation of the experimental diets is shown in Table 1 that it reference NRC fish nutritional needs standards. All diets were come from the University of Sichuan Agricultural Animal Care Advisory Committee and individually blended in a mixer. The mixture was produced the noodle-like diets when added to water. By atomic absorption spectrometry determination of zinc and copper content in the mixture. For the feeding trail, each experimental diet was fed to three replicate groups of fish initially at 2-3% body weight per day.

Design of experiment and sample collection: Picking over 540 fish with an average initial weight of 3.3±0.1 g were randomly assigned to each of 18 experimental aquaria. Six experimental diets were formulated to give 0, 15, 30, 60, 90 and 120 mg kg⁻¹ zinc. After feeding for 80 days, 27 fish were collected from each group 24 h after the last feeding and drew blood from fish tail vein and the hepatopancreas and intestine were removed. The blood samples were centrifuged at 3200×g and 4°C for 10 min and the supernatant conserved. Tissue samples were homogenized in 10 volumes (w/v) of ice-cold physiological saline centrifuged at 3200×g and 4°C for 10 min and the supernatant conserved. At the end of the feeding trial, 30 fish were collected from each group and injected *Aeromonas hydrophila* (*A. hydrophila*) 0.15 mL/body (*A. hydrophila* initially isolated from dropsy and septicemic conditions of fish *Channa striatus* and *Clarias batrachus*. *A. hydrophila* was cultivated for 24 h at 28°C in broth medium. After 24 h of growth, the bacteria was washed and suspended in PBS (pH 7.2). Fish were observed for a period of 7 days and the Lethal Dose (LD50) was determined and final concentration was adjusted to 3.7×10⁹ CFU mL⁻¹ whereas) in the fish stomach to statistics death rates for 15 days between sooner and later of every day (Table 2).

Table 2: Experiment design

| Items | Groups | | | | | |
|---|--------|----|----|----|----|-----|
| | A | B | C | D | E | F |
| Replicates | 3 | 3 | 3 | 3 | 3 | 3 |
| Numbers/tank | 30 | 30 | 30 | 30 | 30 | 30 |
| Adding levels ¹ (mg kg ⁻¹) | 0 | 15 | 30 | 60 | 90 | 120 |

¹Zinc supplementation

Detection method

Growth performance:

$$\text{Percent weight gain} = \frac{\text{Weight gain} \times 100}{\text{Initial weight}}$$

$$\text{Specific growth rate} = \frac{(\ln W_t - \ln W_o) \times 100}{t}$$

Where:

W_t = Final weight

W_o = Initial weight

t = 80 days

$$\text{Relative fatness} = \frac{W}{L^3} \times 100$$

Where:

W = Final weight

L = Body length

$$\text{Feed efficiency} = \frac{\text{Diet intake}}{\text{Weight gain}}$$

The activities of protease and lipase were determined by using the method of Folin-Phenol Reagent Method and Iodine-Starch Colorimetric Method. The activities of amylase was determined by using the method of Polyvinyl Alcohol Olive Oil Method. All kits were come from Nanjing Built Biological Engineering Institute of China.

Non-specific immunity: The hemo-ratio erythrocyte was determined by using the method of Luolin (Ling *et al.*, 2001), AKP and lysozyme activity were measured by using the kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Antioxidant capacity: The Total Antioxidant enzymes (T-AOC), Catalase (CAT), Superoxide Dismutase (SOD), CuZn Superoxide Dismutase (CuZn-SOD) activities and Malondialdehyde (MDA) in plasma and hepatosomatic were determined with a spectrophotometer (UV-2100, Shanghai Jinhua Technology Instrument Co., Ltd. Shanghai, China), respectively. Antioxidant-Related Parameter Detection kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Statistical analyses: Percentage data were subjected to arcsine transformation before statistical analysis. All data were subjected to one-way Analysis of Variance (ANOVA) followed by the use of Tukey's Method to determine significant differences among treatment groups. The level of significance was set to $p < 0.05$. The level of great significance was set to $p < 0.01$. Statistical analysis was performed using the SPSS 20.0 suite for Windows (SPSS Inc., Chicago, Illinois, USA).

RESULTS AND DISCUSSION

Growth performance: Dietary zinc supplementation generally increased percent weight gain, specific growth rate, relative fatness and feed efficiency in Table 3. Percent weight gain and specific growth rate increased the highest with dietary zinc supplementation levers than group A. However, in the group C, the percent weight gain increased the highest significantly than group A and relative fatness increased great significantly than group A. Feed efficiency of group C and group D were significantly reduced than group A.

Digestive enzyme activity in hepatopancreas and intestine increased significantly with dietary zinc supplementation levers than group A in Table 4. Protease and amylase activity of group C in hepatopancreas increased higher than group B, E and F but it was not increased significantly between group C and group D. Lipase activity of group C in hepatopancreas were increased the highest than group E and F but it was increased higher than group B and D. Protease, amylase and lipase activity of group C in intestine were increased the highest than group B and F.

Non-specific immunity: The hemo-rate erythrocyte significantly higher than group A but there were no significant differences for group A and F in Table 5. The plasma lysozyme activity increased steadily when the supplemental zinc was increased by up to group D and then declined with further addition, no significant difference was observed between group C and D. Similarly, the plasma AKP activity increased steadily when the supplemental zinc was increased by up to group C and then declined with further addition but no significant difference was observed to group D, E and F. The challenge test showed that long-term oral administration of zinc-supplemented diets generally enhanced protection against *A. hydrophila* infection. However, significantly higher post-challenge survival rates were only observed in group C.

Antioxidant-related parameters: Dietary zinc supplementation generally increased the plasma and hepatopancreas antioxidant enzyme activities in Table 6. The plasma T-AOC activity was significantly the highest between group B and C, there were no significant difference was observed among group A, D and F. The plasma CAT activity increased steadily when the supplemental zinc was increased by up to group C and then declined with further addition. The plasma SOD and CuZn-SOD activity of group B and C were significantly the highest than group A but there were no significant difference. The plasma MDA content decreased steadily when the supplemental zinc was increased by up to group D and then increased with further addition. The hepatopancreas T-AOC and CAT activity increased steadily when the supplemental zinc was increased by up

Table 3: Effects on growth of *Misgurnus anguillicaudatus* by zinc

| Items | Initial weight (g/body) | Final weight (g/body) | Percent weight gain (%) | Specific growth rate (% day ⁻¹) | Relative fatness (%) | Feed efficiency (%) |
|---------|----------------------------|--------------------------|----------------------------|--|-------------------------|-------------------------|
| Group A | 3.40±0.02 | 6.23±0.28 ^a | 94.56±20.15 ^{Aa} | 0.809±0.027 ^{Aa} | 2.75±0.31 ^a | 3.17±0.19 ^b |
| Group B | 3.41±0.03 | 7.18±0.36 ^b | 110.67±19.99 ^{Bb} | 0.910±0.024 ^{Bb} | 2.78±0.19 ^a | 2.59±0.24 ^{ab} |
| Group C | 3.31±0.01 | 7.63±0.69 ^b | 130.61±25.14 ^{Bd} | 1.015±0.025 ^{Bc} | 4.10±0.84 ^b | 2.26±0.95 ^a |
| Group D | 3.31±0.01 | 7.31±0.94 ^b | 120.97±28.49 ^{Bc} | 0.981±0.016 ^{Bc} | 3.00±0.60 ^a | 2.20±0.58 ^a |
| Group E | 3.42±0.03 | 7.28±0.76 ^b | 112.81±21.35 ^{Bb} | 0.909±0.030 ^{Bb} | 3.63±0.46 ^{ab} | 2.76±0.54 ^{ab} |
| Group F | 3.31±0.01 | 7.05±0.70 ^b | 112.99±25.05 ^{Bb} | 0.913±0.028 ^{Bb} | 3.40±0.29 ^{ab} | 2.88±0.10 ^{ab} |

Table 4: Effects of zinc on digestive enzyme activity in hepatopancreas and intestine of *Misgurnus anguillicaudatus*

| Items | Groups | | | | | |
|-------------------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| | A | B | C | D | E | F |
| Protease (U mg⁻¹) | | | | | | |
| Hepatosomatic | 625.56±24.63 ^{Aa} | 935.19±47.91 ^{Bb} | 991.45±51.01 ^{Bc} | 1096.12±33.78 ^{Bc} | 945.84±9.75 ^{Bb} | 933.57±44.30 ^{Bb} |
| Intestine | 819.20±34.22 ^{Aa} | 1098.47±44.03 ^{Bb} | 1277.83±15.27 ^{Cd} | 1156.65±45.56 ^{Bc} | 1218.59±27.36 ^{Cd} | 1021.46±44.03 ^{Bb} |
| Lipase (U g⁻¹) | | | | | | |
| Hepatosomatic | 70.35±6.63 ^{Aa} | 144.38±13.72 ^{Cc} | 162.52±28.78 ^{Cd} | 148.79±19.25 ^{Cc} | 111.41±19.91 ^{Bb} | 105.52±12.38 ^{Bb} |
| Intestine | 69.64±7.35 ^{Aa} | 99.07±18.14 ^{Bc} | 118.14±8.74 ^{Cd} | 115.92±9.51 ^{Cd} | 94.96±7.96 ^{Bc} | 83.31±16.45 ^{ABb} |
| Amylase (U mg⁻¹) | | | | | | |
| Hepatosomatic | 2.57±0.05 ^{Aa} | 5.75±0.65 ^{Bb} | 7.33±1.79 ^{Bc} | 6.97±0.42 ^{Bc} | 4.57±0.20 ^{Bb} | 4.43±0.33 ^{Bb} |
| Intestine | 1.46±0.06 ^{Aa} | 2.06±0.13 ^{Ab} | 5.28±1.26 ^{Cd} | 4.36±0.39 ^{Cc} | 1.76±1.38 ^{Ab} | 1.84±0.40 ^{Ab} |

Values in the same row with different superscripts are significantly different ($p < 0.05$). With different capital letter superscripts mean significant difference ($p < 0.01$). While with same small letter superscripts or no letter superscripts mean no significant ($p > 0.05$)

to group C and then declined with further addition. The hepatopancreas SOD activity was significantly the highest between group B and C. The hepatopancreas CuZn-SOD activity was significantly the highest between group C and D compared to group A but there were no significant difference between group A and F. The hepatopancreas MDA content decreased steadily when the supplemental zinc was increased by up to group C and then increased with further addition.

Growth: Zinc is one of the major micronutrient and fish is considered as a low zinc food which provides $<1 \mu\text{M}$ of zinc per gram of protein. It is evident from the literature that zinc plays a critical role in the activity of the enzymes in fish (Maret, 2005). The present study which was based on a dose response design, showed that percent weight gain, specific growth rate, relative fatness and digestive enzyme activity of fish all increased with increasing dietary zinc supplementation up to a point and there was no increase thereafter with further increases in dietary zinc supplementation. This agrees with the study about other fish (Lina, 2009; Wanquan and Aijie, 1999; Gatlin and Wilson, 1983; Ogino and Yang, 1978; Wu, 2007; Zhang *et al.*, 2007) in which effect of dietary zinc

supplement were found on the growth. In this study, percent weight gain and specific growth were significantly increased in response to high dietary zinc. Digestive enzyme activity of group C was increased higher than group A. It is well documented that the optimal dietary zinc content for optimum *Misgurnus anguillicaudatus* growth performance and digestive ability. Digestive ability is correlated with digestive enzyme activity as nutrients are digested by these enzymes (Zhou *et al.*, 2001). The enzyme activities are affected by dietary intake. This study have confirmed that zinc is carboxy peptide enzyme, protease, amylase, lipase, alkaline phosphatase and other forms of enzyme activity of the essential cofactor such as carboxy peptide enzyme catalytic require the participation of a zinc ions. The experiment shows that the zinc can improve digestive enzymes activity, thus, to improving its to protein, fat and carbohydrate digestion ability. Similarly, the study have found that dietary zinc supplementation in water increased aspartate transaminases activities in the process of metabolism (Zhang *et al.*, 2004). The study have found that zinc supplementation in the die can increase Na^+ , K^+ -ATPase activities (Lina, 2009). The report that zinc was participate in the synthesis of nucleic acids and proteins through DNA polymerase and RNA polymerase (Wangbao *et al.*, 2007; Pang and Zhang, 2002). This result is evidence that zinc supplementation in the die can improve the ability of aquatic animals to digestion and absorption of nutrients, participate in the synthesis of nucleic acid and protein, energy metabolism, so as to promote the growth of aquatic animals.

Immune response: Fish are held in aquaculture pens, live in an environment where they are constantly exposed to various stress factors such as handling, crowding and infection leading to immune depression and outbreaks of

Table 5: Effects of zinc on non-specific immunomodulation of *Misgurnus anguillicaudatus*

| Items | The hemo-rate erythrocyte | Lysozyme ($\mu\text{g mL}^{-1}$) | AKP ($\text{U } 100 \text{ mL}^{-1}$) | Survival rate (%) |
|---------|--------------------------------|------------------------------------|---|--------------------------------|
| Group A | 17.50 \pm 1.29 ^a | 4.36 \pm 0.14 ^{aa} | 140.79 \pm 28.44 ^{aa} | 36.67 \pm 0.00 ^{aa} |
| Group B | 24.75 \pm 1.29 ^c | 5.00 \pm 0.32 ^{ab} | 255.18 \pm 72.56 ^{bb} | 50.00 \pm 0.00 ^{bc} |
| Group C | 22.50 \pm 2.38 ^b | 7.50 \pm 0.58 ^{bd} | 354.75 \pm 20.45 ^{cd} | 66.67 \pm 0.00 ^{cd} |
| Group D | 21.50 \pm 2.08 ^b | 8.25 \pm 0.95 ^{bd} | 316.78 \pm 35.21 ^{cc} | 53.33 \pm 0.00 ^{bc} |
| Group E | 21.50 \pm 2.08 ^b | 6.00 \pm 0.42 ^{bc} | 302.77 \pm 16.75 ^{cc} | 40.00 \pm 0.00 ^{ab} |
| Group F | 18.00 \pm 2.16 ^{ab} | 6.50 \pm 0.77 ^{bc} | 307.71 \pm 21.36 ^{cc} | 40.00 \pm 0.00 ^{ab} |

Values in the same row with different superscripts are significantly different ($p < 0.05$). With different capital letter superscripts mean significant difference ($p < 0.01$). While with same small letter superscripts or no letter superscripts mean no significant ($p > 0.05$)

Table 6: Effect of zinc on the antioxidant capability in plasma and hepatopancreas of *Misgurnus anguillicaudatus*

| Items | Groups | | | | | |
|---|--------------------------------|---------------------------------|---------------------------------|--------------------------------|--------------------------------|--------------------------------|
| | A | B | C | D | E | F |
| T-AOC (U mL^{-1}) | | | | | | |
| Plasma | 4.93 \pm 0.26 ^{aa} | 11.10 \pm 1.91 ^{bc} | 15.61 \pm 2.13 ^{bd} | 5.55 \pm 0.65 ^{aa} | 7.40 \pm 0.87 ^{ab} | 4.32 \pm 0.36 ^{aa} |
| Hepatopancreas | 1.22 \pm 0.63 ^{aa} | 3.15 \pm 0.16 ^{bb} | 3.34 \pm 0.32 ^{bc} | 2.75 \pm 0.25 ^{bb} | 2.25 \pm 0.08 ^{bb} | 2.47 \pm 0.83 ^{bb} |
| CAT (U mL^{-1}) | | | | | | |
| Plasma | 17.80 \pm 1.47 ^{aa} | 25.56 \pm 3.45 ^{ac} | 31.44 \pm 2.01 ^{bd} | 21.59 \pm 2.57 ^{ab} | 22.72 \pm 2.32 ^{ab} | 21.91 \pm 1.56 ^{ab} |
| Hepatopancreas | 22.57 \pm 0.12 ^{aa} | 26.65 \pm 0.77 ^{ab} | 34.52 \pm 2.62 ^{bc} | 24.77 \pm 2.46 ^{aa} | 26.34 \pm 3.04 ^{ab} | 24.71 \pm 1.47 ^{aa} |
| SOD (U mL^{-1}) | | | | | | |
| Plasma | 65.84 \pm 3.88 ^{aa} | 94.96 \pm 2.16 ^{cd} | 93.27 \pm 3.57 ^{cd} | 86.10 \pm 1.93 ^{bc} | 72.46 \pm 2.04 ^{ab} | 77.66 \pm 5.26 ^{ab} |
| Hepatopancreas | 74.00 \pm 2.33 ^{aa} | 131.46 \pm 7.83 ^{cd} | 121.45 \pm 3.95 ^{cc} | 94.77 \pm 4.22 ^{bb} | 92.28 \pm 2.57 ^{bb} | 73.13 \pm 4.62 ^{aa} |
| CuZn-SOD (U mL^{-1}) | | | | | | |
| Plasma | 36.05 \pm 2.45 ^{aa} | 62.82 \pm 6.97 ^{bc} | 61.45 \pm 3.91 ^{bc} | 45.51 \pm 8.59 ^{ab} | 39.62 \pm 7.61 ^{aa} | 42.05 \pm 5.73 ^{ab} |
| Hepatopancreas | 44.01 \pm 7.93 ^{aa} | 47.10 \pm 7.28 ^{ab} | 50.91 \pm 2.48 ^{ab} | 73.83 \pm 3.59 ^{bc} | 48.67 \pm 6.10 ^{ab} | 45.52 \pm 3.76 ^{aa} |
| MDA (nmol mL^{-1}) | | | | | | |
| Plasma | 13.50 \pm 0.71 ^{cd} | 11.00 \pm 0.50 ^{abc} | 11.25 \pm 2.22 ^{bc} | 9.00 \pm 2.58 ^{aa} | 11.67 \pm 1.13 ^{bc} | 10.25 \pm 2.79 ^{ab} |
| Hepatopancreas | 9.17 \pm 2.05 ^{cd} | 7.36 \pm 1.78 ^{ab} | 5.56 \pm 0.67 ^{aa} | 6.94 \pm 1.49 ^{ab} | 8.26 \pm 1.61 ^{bc} | 8.40 \pm 1.06 ^{bc} |

Values in the same row with different superscripts are significantly different ($p < 0.05$). With different capital letter superscripts mean significant difference ($p < 0.01$). While with same small letter superscripts or no letter superscripts mean no significant ($p > 0.05$)

infections (El-Boshy *et al.*, 2010). Zinc is fish necessary trace elements, almost all of the immune response performance depends on enough zinc content (Wangbao *et al.*, 2007; Pang and Zhang, 2002). Red blood cells surface of C3b receptors, C3b can adhesion molecule analyte complex and submit it for neutrophils and macrophages, enhance the phagocytosis of phagocytic cells. Meanwhile red blood cells contain rich SOD enzyme, the release of SOD can protect the phagocytosis in phagocytic cells antigens produced in the process of oxygen free radicals to ruin the oxidative damage of cell membrane thus enhance phagocytic cells in the scavenging effect of bacterium of cause of disease (Feng, 2002; Wenbin, 2004). Rongier report that $0.2 \times LD_{50}$ concentration of Zn^{2+} can promote zebrafish lymph cell division and the body's immune function (Rougier *et al.*, 1996). The study found that dietary zinc supplementation improves the hago-rate erythrocyte of *Misgurnus anguillicaudatus*. In the present study, the lysozyme and AKP activity increased steadily when the supplemental zinc was increased by up to 15.3 or 40.78 mg kg⁻¹ of crap (Lina, 2009). The present study indicated that supplemental zinc was increased significant lysozyme and AKP activity in plasma of *Misgurnus anguillicaudatus*. The AKP a typical lysosomal enzyme and involves in killing and digesting microbial pathogens during immune responses, the molecules containing 1 of Zn^{2+} can catalytic hydrolysis of phosphatase and play a role in the process of the body's defense (Rongier *et al.*, 1996; Changfu and Yong, 2007). Lysozyme can kill bacteria by enzymatic hydrolysis pathogens of cell wall polysaccharide so the level of lysozyme and activity directly related to the immune system and healthy of fish. By increasing serum lysozyme and AKP activity to enhance *Misgurnus anguillicaudatus* capability of sterilization and further strengthen loach nonspecific immunity.

Antioxidant activities: The body's defense system of the antioxidation ability strong and the weak is closely related to health. In addition, multiple types of antioxidants (e.g., T-AOC, CAT, SOD and MDA) are needed to maintain the complex immune system of fish (Wangbao *et al.*, 2007; Pang and Zhang, 2002). The antioxidant capacity includes enzymatic and non-enzymatic antioxidant activities. Antioxidant enzymes include T-AOC, CAT, SOD and MDA that constitute the first line of the enzymatic defence mechanism against free radicals in organisms. SOD catalyses dismutation of superoxide radicals to hydrogen peroxide and oxygen; CAT catalyses breakdown of hydrogen peroxide to water and molecular oxygen (Tovar-Ramirez *et al.*, 2010). The study was

found that the SOD and CAT activity in plasm and hepatopancreas of crap increased steadily when the supplemental zinc was increased (Lina, 2009). In the present study, the T-AOC, CAT, SOD and CuZn-SOD activity in plasm and hepatopancreas of *Misgurnus anguillicaudatus* increased steadily when the supplemental zinc was increased. The group C of T-AOC activity was significantly the highest group A. The T-AOC ability is the comprehensive reflection of the body, the enzyme antioxidant system and antioxidant enzyme system to complete the antioxidant effect. In the present study, the T-AOC, CAT, SOD and CuZn-SOD activity in plasm and hepatopancreas of *Misgurnus anguillicaudatus* increased steadily when the supplemental zinc was increased. In the present study was found that zinc through improve antioxidant enzyme system of CAT and SOD and CuZn-SOD activity to improve the total antioxidant capacity, make *Misgurnus anguillicaudatus* defense ability enhancement. CuZn-SOD is a parting of SOD. In the present study, this trial is the first to determination of aquatic animals with SOD and CuZn-SOD vigor from experiment showed that zinc additives can increase SOD and CuZn-SOD vitality and there are becoming to path linear relationship. Zinc can restrain of the mitochondrial cytochrome P450 enzyme system to produce free radicals and reducing iron ions to enter the cell and resist the free radical initiated in light of the chain reaction of catalysis through participating in composition CuZn-SOD inhibition NADPH-cytochrome C Reductase and activating the GSH-Px (Xiaoli *et al.*, 2004; McCord and Fridovich, 1969). MDA production was a well-known oxidation process resulting from the peroxidation of membrane polyunsaturated fatty acid, influencing cell membrane fluidity as well as the integrity of biomolecules and was an important indicator of lipid peroxidation (Almroth *et al.*, 2005). In the present study, *Misgurnus anguillicaudatus* fed the optimal dietary Zn level of the diet reduced hepatic and plasm MDA level. This study show that adequate dietary Zn supplementation can improve the total antioxidant response and reduce lipid peroxidation reaction speed, clear the MDA of body in the commutation period and avoid the body damage when it is appearing.

CONCLUSION

The study demonstrate that dietary zinc supplementation improves *Misgurnus anguillicaudatus* growth performance and digestion ability, promoted non-specific immunity and antioxidant ability. The optimal dietary zinc supplementation in *Misgurnus anguillicaudatus* was 30 mg kg⁻¹ diet.

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