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Comparison of chelated zinc and zinc sulfate as zinc sources for growth and immune response of shrimp (*Litopenaeus vannamei*)

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ABSTRACT

Zinc methionine (ZnMet), zinc lysine (ZnLys), zinc glycine (ZnGly) and zinc sulfate ($ZnSO_4 \cdot H_2O$) were evaluated as dietary zinc sources for *Litopenaeus vannamei*. Three Zn–amino acid complexes with a molar amino acid to Zn ratio of 2:1 were compared to Zn sulfate using a casein-based purified diet. Five groups with four replicates of shrimps (mean weight 0.72 ± 0.02 g) were given a basal diet either unsupplemented (control) or supplemented with 30 mg Zn kg^{-1} sulfate ($ZnSO_4 \cdot H_2O$) or the organic sources respectively, for 12 weeks. Results showed that the source of Zn affects shrimp growth, survival and immune parameters. Shrimp fed diets with organic zinc supplementation produced significantly higher growth, survival and immune parameters than $ZnSO_4$ treatment. Shrimp supplemented with ZnMet had the highest weight gain and immune parameters. However, there were no significant differences in weight gain, survival, total hemocyte counts, phagocytotic activity, PO, AKP and SOD between the ZnLys and ZnGly groups. Results suggest that Zn from ZnMet was a better source than the other zinc forms.

1. Introduction

Zinc (Zn) is an essential nutrient that is required in humans and animals for many physiological functions including growth, development, reproduction and immune function (Watanabe et al., 1997). Depending on the doses and the chemical forms of Zn, it can act as nutrients, antioxidants, or even toxicants (Lemire et al., 2008). Zinc functions as a cofactor in several enzyme systems and is a component of a large number of metalloenzymes, which include carbonic anhydrase, alkaline phosphatase and DNA polymerases (NRC, 2011). However, deficient or excessive zinc has been reported to affect the fish morphology, biochemical processes and growth (Watanabe et al., 1997). Like other heavy metals, deficient or excessive zinc could also exert inhibitory effects on immune responses and increase the severity of infections in humans and animals (Shankar and Prasad, 1998). The dietary Zn supply to fish often largely exceeds their actual requirements. Poorly absorbed, Zn is highly concentrated in nature and may cause environmental pollution in areas of intensive aquaculture production (NRC, 2011). Absorption of trace elements often limits their utilization. One of the factors that affect mineral absorption and utilization is their chemical form. Hence, mineral sources with higher bioavailability should be considered in feed formulation.

Due to higher bioavailability compared to inorganic salts, chelated minerals as animal feed supplements have attracted considerable attention of the feed manufacturers and the animal producers as a means of improving animal performance (Ashmead, 1992; Wang and Lovell, 1997). Nowadays, chelated minerals are widely used in the livestock and poultry industries (Puchala et al., 1999; Swiatkiewicz et al., 2001; Wedekind et al., 1992). Some studies regarding chelated minerals have already been conducted in either fishes (Apines-Amar et al., 2004; Paripatananont and Lovell, 1995; Satoh et al., 2001; Wang and Lovell, 1997) or abalone (Tan and Mai, 2001), and beneficial effects on growth performance or immunity have been reported. As for *Litopenaeus vannamei*, there is little known about the effectiveness of any chelated minerals including organic Zn sources at present.

The white shrimp (*L. vannamei*) is one of the most commercially cultured shrimp species in South China (FAO, 2010). The shrimp culture industry has often suffered economic losses attributed to outbreaks of infectious viral and bacterial diseases. The establishment of health management regimes and the selection of shrimp that are more resistant to diseases are facilitated through the characterization of effectors of the immune system. Our understanding on the role of dietary nutrients on shrimp health is largely based on nutrients such as minerals. However, potential benefits of organic Zn complexes on the immune function of shrimp have not been critically evaluated. Thus, the current study was designed to evaluate the application of different zinc sources, including inorganic Zn (zinc sulfate) and organic Zn (ZnMet, ZnLys and ZnGly), as feed additives in diets for *L. vannamei*.

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2. Materials and methods

2.1. Experimental diets

The basal diet formulation is given in Table 1. It was formulated with purified ingredients to provide 41% crude protein from casein and gelatin and 6.5% crude lipid from soybean oil and menhaden fish oil (1:2), which were sufficient to support optimal growth (Roy et al., 2006). The composition of mineral premix was modified according to Kureshy and Davis (2002) without zinc supplementation. The vitamin mixture was similar to that used by Kureshy and Davis (2002). Zinc methionine (ZnMet), zinc lysine (ZnLys), zinc glycine (ZnGly) and zinc sulfate ($ZnSO_4 \cdot H_2O$) were used as dietary zinc sources. The organic source of Zn was developed by Calcialiment (Changsha Xingjia Biotechnology Share Co., Ltd. Hunan, China) and is a Zn–amino acid complex with a molar amino acid to Zn ratio of 2:1. Zinc from either sulfate (feed-grade; $ZnSO_4 \cdot H_2O$) or the organic source was added into the basal diet at levels of 30 mg Zn kg^{-1} diet according to NRC (2011), respectively, to prepare the four experimental diets. The control groups were basal diet only. The Zn concentrations of the basal diets were analyzed by flame photometry after wet decomposition according to AOAC (1995) and found to be 6.5 mg Zn/kg diet.

The ingredients were ground in a Hammer mill until they passed through a 60-mesh screen. Experimental diets were prepared by thoroughly mixing the dry ingredients with oil and then adding cold water until a stiff dough resulted. This was then passed through a mincer with die and the resulting "spaghetti-like" strings were dried using an electrical fan at 40 °C. After drying, the diets were broken up and sieved into convenient pellet sizes and stored at −20 °C until being used.

2.2. Experimental animals

Healthy white shrimp, *L. vannamei*, were obtained from a commercial farm in Zhanjiang, Guangdong, China, and acclimated in a re-circulated seawater system for 2 weeks prior to the feeding trial. Two thousand shrimps (initial mean weight 0.72 ± 0.02 g) were randomly distributed to five treatments and each treatment had four replicates. Each 400-l cylindrical fiberglass tank with 100 shrimps was used as a replicate. The shrimps were fed to apparent satiation four times a day at 06:00, 12:00, 16:00 and 20:00. For each time, feed remains and feces of shrimp can be removed by the water system. During the 12-week feeding trial, water temperature was maintained at 28 °C–30 °C, salinity 30–32 ppt, and pH 7.8–8.2. The zinc concentration in the seawater flowing into the rearing system was 4.5 μg/l determined by ICP-OES ($n = 3$). At the termination of the experiment, the shrimps were fasted for 24 h before harvest. The shrimp were weighed and counted.

Table 1
Ingredients and proximate composition of the basal diet.

Ingredients	% (dry weight)
Vitamin-free casein (Sigma, St. Louis, MD, USA)	40.0
Gelatin (Sigma)	6.0
Dextrin (Shanghai Chemical, Shanghai, China)	30.0
Carboxymethylcellulose (Shanghai Chemical)	5
SO/MFO (Food Grade)[a]	6.0
Cholesterol (Shanghai Chemical)	0.5
Vitamin premix[b]	2.0
Zinc-free mineral premix[c]	0.5
a-Cellulose (Sigma)	10

[a] Soybean oil: menhaden fish oil = 1:2.
[b] Vitamin premix (g/kg premix): thiamin HCL 0.5, riboflavin 3.0, pyridoxine HCL 1.0, DL Ca-pantothenate 5.0, nicotinic acid 5.0, biotin 0.05, folic acid 0.18, vitamin B_{12} 0.002, choline chloride 100.0, inositol 5.0, menadione 2.0, vitamin A acetate (20,000 IU/g) 5.0, vitamin D (400,000 IU/g) 0.002, DL-alpha-tocopheryl acetate (250 IU/g) 8.0, L-ascorbyl-2-monophosphate (35% active C) 25.0, and alpha-cellulose 840.266.
[c] Zn-free mineral premix (g/100 g premix): cobalt chloride 0.004, cupric sulfate pentahydrate 0.250, ferrous sulfate 4.0, magnesium sulfate heptahydrate 28.398, manganous sulfate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, and zeolite 66.621.

2.3. Sample collection

After being fasted for 24 h, hemolymph (100 μl) was withdrawn from the ventral sinus of each shrimp into a 1-ml sterile syringe containing 200 μl anticoagulant solution (30 mM trisodium citrate, 10 mM EDTA, 0.34 mM sodium chloride 0.12 mM glucose, adjust pH to 7.55 and osmotic pressure to 780 mOsm/kg). The hemolymph from six shrimps in one tank was pooled. A 1-ml anticoagulant-hemolymph sample was centrifuged at 700 ×*g* at 4 °C for 10 min, and supernatant was used to measure phenoloxidase (PO) activity, superoxide dismutase (SOD) activity and alkaline phosphatase (AKP) activities. About 500 μl anticoagulant-hemolymph was used to measure total hemocyte count (THC) and phagocytic activity of hemocytes.

2.4. Survival and growth performance

At the termination of the experiment, the shrimps were fasted for 24 h before harvest. Total number was counted and mean body weight of shrimp was measured. Based on recording the weight of shrimp and counting the number of shrimps, weight gain, feed conversion ratio (FCR) and survival were calculated using the following equations:

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

$$\text{SGR} = 100 \times [\ln \text{final weight} - \ln \text{initial weight}] \div \text{total duration of the experiment}$$

$$\text{FCR} = \text{feed given}(\text{dry weight}) \div \text{weight gain}(\text{wet gain})$$

$$\text{Survival}(\%) = (\text{final number of shrimps} \div \text{initial number of shrimps}) \times 100.$$

2.5. Immunological assays

2.5.1. Total hemocyte count (THC)

A drop of the anticoagulant-hemolymph was placed on a Buker hemocytometer to measure total hemocyte count (THC) under optical microscope (XPS-BM-2GA, Shanghai BM Optical Institution Manufacture CO. LTD.). The hemocytes were counted manually in all 25 squares (0.1 mm^3).

2.5.2. Phenoloxidase (PO) activity

Phenoloxidase (PO) activity was estimated spectrophotometrically by recording the formation of dopachrome using L-3,4 dihydroxyphenylalanine (L-DOPA; Sigma, USA) as substrate according to Hernández-López et al. (1996). Briefly, 50 μl hemolymph supernatant was incubated with 50 μl trypsin (0.1% in cacodylate buffer: 0.45 M sodium chloride, 0.10 M trisodium citrate, 0.01 M sodium cacodylate, pH 7.0) in 96 well microplate at 25 °C for 10 min, and then 50 μl L-DOPA (0.3% in cacodylate buffer) was added. The absorbance value was read every 2 min in microplate reader (Model Multiskan spectrum, Thermo, MA, Waltham, USA) at 490 nm for 20 min. Enzyme activity was expressed as the change in absorbance per minute per milliliter hemolymph supernatant.

2.5.3. Phagocytic activity

Phagocytotic activity (PA) was determined according to Itami et al. (1994). Collected shrimp hemocytes were rinsed with shrimp saline and the viable cell number adjusted to 1×10^6 cells/ml. The 200 μl cell suspension of shrimp hemocytes was inoculated into a