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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

Table 2
Growth response and survival of *L. vannamei* fed different zinc sources at the end of 12 weeks of feeding trial (means ± SEM).

Parameters	Diet no. (dietary zinc sources)				
	Diet 1 (Control)	Diet 1 ($ZnSO_4 \cdot H_2O$)	Diet 2 (ZnMet)	Diet 3 (ZnLys)	Diet 4 (ZnGly)
Initial weight (g)	0.73 ± 0.01	0.71 ± 0.03	0.72 ± 0.02	0.72 ± 0.02	0.72 ± 0.01
Final weight (g)	5.34 ± 0.04 ^{d}	6.48 ± 0.08 ^{c}	10.77 ± 0.15 ^{a}	7.93 ± 0.18 ^{b}	8.54 ± 0.55 ^{b}
Weight gain (%)	625.1 ± 16.34 ^{d}	830.4 ± 34.64 ^{c}	1394.8 ± 31.68 ^{a}	1024.2 ± 30.32 ^{b}	1092.4 ± 93.82 ^{b}
FCR	1.25 ± 0.03 ^{a}	1.16 ± 0.02 ^{b}	1.03 ± 0.05 ^{c}	1.07 ± 0.08 ^{c}	1.06 ± 0.04 ^{c}
Survival (%)	57.7 ± 1.23 ^{c}	76.2 ± 1.52 ^{a}	94.7 ± 2.16 ^{a}	91.6 ± 1.75 ^{a}	92.3 ± 1.69 ^{a}

Means ± SEM having the same letter in the same row are not significantly different at $P < 0.05$.

cover slip. After 20 min, the cell suspension was removed and rinsed with shrimp saline three times. Heat-killed yeast preparation (Baker's yeast, Type II, Sigma, USA, 1×10^8 cells/ml) was added and incubated for 2 h. Then unattached cells were rinsed with shrimp saline five times. After air-drying, the slides were fixed in methanol and stained with Giemsa solution (Sigma, St. Louis, MO, USA). Slides were mounted with Permount slide mounting fluid. Two hundred hemocytes were counted for each sample under the microscope. Phagocytic activity, defined as percentage phagocytosis was expressed as

$$\text{Percentage phagocytosis} = \text{phagocytic hemocytes} \div \text{total hemocytes} \times 100.$$

2.5.4. Alkaline phosphatase (AKP)

Alkaline phosphatase (AKP) activity was estimated spectrophotometrically using *p*-nitrophenyl phosphate (Sigma, USA) as substrate following the modified method described by Gonzalez et al. (1994). A sample of 100 µl of crude enzyme solutions was incubated for 30 min at 37 °C with 2.0 ml of substrate (*p*-nitrophenyl phosphate in glycine–NaOH buffer-pH 9 for alkaline phosphatase). Then 2.9 ml of 0.1 N NaOH was added and the absorbance measured spectrophotometrically at 405 nm and the activity expressed as mg/ml *p*-nitrophenyl released.

2.5.5. Serum superoxide dismutase

Superoxide dismutase (SOD) activity was measured by its ability to inhibit superoxide anion generated by xanthine and xanthine oxidase reaction system according to Wang and Chen (2005) using an SOD detection kit (Nanjing Jiancheng Bioengineering Institute, China). The optical density was measured at 550 nm. One unit of SOD was defined as the amount required for inhibiting the rate of xanthine reduction by 50% in 1 ml reaction system. Specific activity was expressed as SOD unit per milliliter serum.

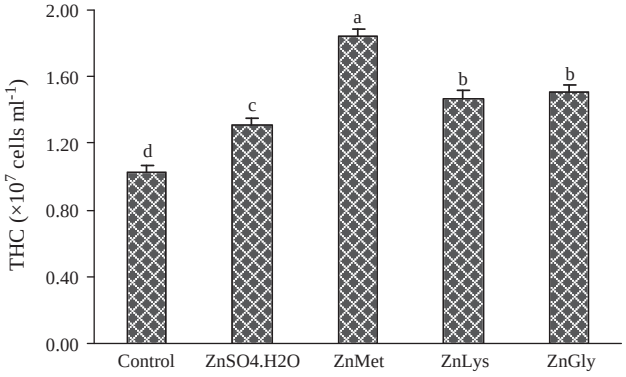

Fig. 1. Total hemocyte count (THC) of *L. vannamei* fed different zinc sources. Data are expressed as mean (SEM). Means in the same column sharing the same superscript letter are not significantly different as determined by Tukey's test ($P > 0.05$). $n = 20$.

2.6. Challenge test

The *Vibrio harveyi* strain was originally isolated from a diseased *L. vannamei*. The seven day LD_{50} was determined by intraperitoneal injection of 100 shrimps with graded doses of *V. harveyi* (10^4, 10^5, 10^6, 10^7 and 10^8 cfu/shrimp) at 24 °C, and the result showed that LD_{50} on day 7 was 10^6 cfu/shrimp.

Challenge tests were conducted in triplicate with 12 shrimps per replicate. Each shrimp was injected intraperitoneally with 0.3 ml PBS containing 2.8×10^6 live *V. harveyi* from a 24 h culture in 2216E medium at 25 °C. The shrimps were then kept in separate 100 l glass aquaria (12 shrimps each). A total of 240 shrimps (48 × 5) were used for the study. No diet was given to the animals during the test. The shrimps were observed for the presence of disease manifested. Mortality of shrimp in each tank was observed over 14 days, and the average of the triplicate tanks was used to express cumulative mortality and relative percent survival (RPS) values were calculated as follows:

$$\text{RPS} = 100 - [(\text{treatment mortality} \div \text{control mortality}) \times 100].$$

2.7. Statistical analysis

All data were subjected to one way ANOVA (analysis of variance) using SPSS 16.0 for Windows. Differences between the means were tested by Tukey's multiple range tests. Overall significance level = 0.05 and the results are presented as means ± SEM (standard error of the mean).

3. Results

3.1. Growth

After 12 weeks of feeding period, the shrimp fed the diets supplemented with the organic zinc sources tended to have better growth performance. Shrimp supplemented with ZnMet showed the highest final weight and weight gain in comparison with the other groups ($P < 0.05$; Table 2). However, there were no differences among shrimp fed ZnLys

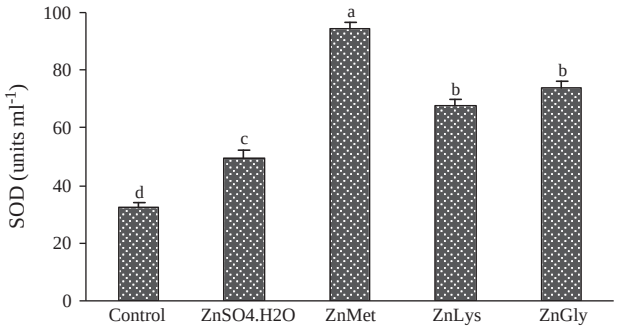

Fig. 2. Superoxide dismutase (SOD) activity of *L. vannamei* fed different zinc sources. Data are expressed as mean (SEM). Means in the same column sharing the same superscript letter are not significantly different as determined by Tukey's test ($P > 0.05$). $n = 20$.

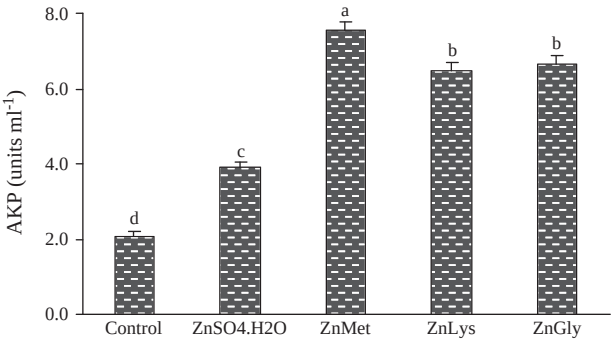

Fig. 3. Alkaline phosphatase (AKP) activity of *L. vannamei* fed different zinc sources. Data are expressed as mean (SEM). Means in the same column sharing the same superscript letter are not significantly different as determined by Tukey's test ($P > 0.05$). $n = 20$.

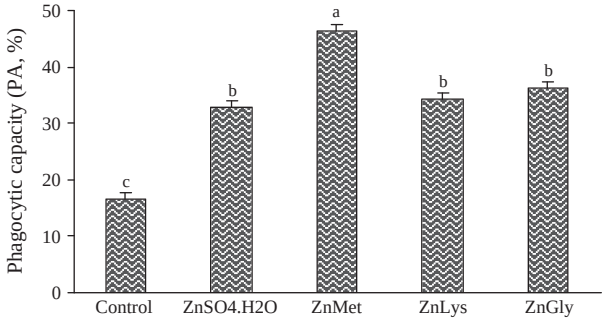

Fig. 5. Phagocytic capacity of *L. vannamei* fed different zinc sources. Data are expressed as mean (SEM). Means in the same column sharing the same superscript letter are not significantly different as determined by Tukey's test ($P > 0.05$). $n = 20$.

and ZnGly. The poorest growth was observed in the shrimps fed the control diet. The greatest improvements in FCR were seen in the organic zinc groups ($P < 0.05$; Table 2). The cumulative survival of the shrimp was significantly affected by dietary organic zinc sources ($P < 0.05$), but no significant differences were found among the organic zinc sources (Table 2).

3.2. Immune response

Shrimp fed diets supplemented with t the organic zinc sources had the highest THC, SOD and AKP, followed by shrimp fed the diets with the zinc sulfate, and lowest in shrimp fed the diets with the control ($P < 0.05$; Figs. 1–3). The THC, SOD and AKP in shrimp fed diets with ZnMet were significantly higher compared with ZnLys and ZnGly ($P < 0.05$; Figs. 1–3). No significant differences in THC, SOD or AKP activity were detected between ZnLys and ZnGly groups (Figs. 1–3).

Shrimp supplemented with zinc exhibited significant increase in PA and PO activity compared with the control ($P < 0.05$; Figs. 4–5), and the ZnMet groups showed the maximum increase ($P < 0.05$) compared with all other groups. On the other hand, no significant differences in PA or PO activity were observed among shrimp fed zinc sulfate, ZnLys and ZnGly (Figs. 4–5).

3.3. Challenge test

The challenge test ($n = 100$ for each dietary treatment) showed that long-time oral administration of the exogenous zinc supplementation enhanced the protection against bacterial infection. The average total mortality rate in shrimp supplemented with zinc was significantly lower than the control group, and the valves in shrimp fed with ZnMet were significantly higher compared with all other groups ($P < 0.05$; Table 3). RPS was lowest in the control group ($P < 0.05$). However, no significant differences in average mortality and RPS were detected among zinc sulfate, ZnLys and ZnGly groups ($P > 0.05$; Table 3).

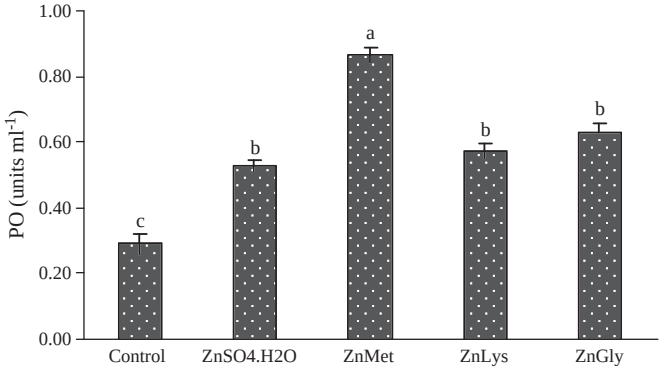

Fig. 4. Phenoloxidase (PO) activity of *L. vannamei* fed different zinc sources. Data are expressed as mean (SEM). Means in the same column sharing the same superscript letter are not significantly different as determined by Tukey's test ($P > 0.05$). $n = 20$.

4. Discussion

The current study demonstrates the benefit of dietary supplementation with amino acid-chelated zinc on the growth of *L. vannamei*. In other aquatic animals, the use of chelated trace elements has resulted in improved growth (Apines-Amar et al., 2004; Paripatananont and Lovell, 1995; Satoh et al., 2001; Tan and Mai, 2001). Micronutrients particularly Zn are known to be essential for growth both in animals and humans (Apines-Amar et al., 2004; Sharif et al., 2012). Deficiency of Zn reduced the levels of IGF-I, growth hormone receptor and growth hormone binding protein mRNA (Clegg et al., 1995; McNall et al., 1995). Like IGF-I, Zn can increase the protein component of bone and play a role in bone growth in collaboration with IGF-I (Ma and Yamaguchi, 2001a,b). In the present study, the higher weight gain in the chelate compared to the sulfate-fed shrimp indicates that the former is a better source of the elements which provide higher bioavailability than the latter. Similarly, in chicks Wedekind et al. (1992), Swiatkiewicz et al. (2001) and Cao et al. (2002) reported a higher bioavailability of Zn from organic sources relative to $ZnSO_4$. Organic zinc sources have been increasingly used in feed industry during the last 10 years. However, when compared to their inorganic forms, there are conflicting results reported regarding bioavailability of these products (Ammerman et al., 1995). Some studies with chicks (Pimentel et al., 1991) and pigs (Revy et al., 2002; Swinkels et al., 1996; Wedekind et al., 1994) indicated no significant differences in the availability of Zn from either sources.

Total number of hemocytes has been used as indicators of health of aquatic animals (Fotedar et al., 2001). Loss and damage of circulating hemocytes would depress the immune ability, increase the susceptibility against pathogens, and even endanger the survival of shrimp (Lorenzon et al., 1999; Yeh et al., 2004). Current study shows that the zinc sources can also alter the hemocyte counts in shrimp. The higher number of hemocytes from shrimps fed with

Table 3
Total average mortality (%) 10 days after challenge with *Vibrio harveyi* in *L. vannamei* fed different zinc sources (means ± SEM).

Diet no. (dietary zinc sources)	Average mortality (%)	RPS (%)
Diet 1 (Control)	69.8 ± 1.6 ^{a}	0 ^{c}
Diet 2 ($ZnSO_4 \cdot H_2O$)	55.6 ± 1.1 ^{b}	22 ^{b}
Diet 3 (ZnMet)	35.3 ± 0.9 ^{c}	49 ^{a}
Diet 4 (ZnLys)	53.7 ± 1.2 ^{b}	23 ^{b}
Diet 5 (ZnGly)	51.8 ± 1.3 ^{b}	26 ^{b}

Data are the means ± SEM from 45 shrimps. Values in any one column not followed by the same superscripts are significantly different at $P < 0.05$.

ZnMet supplemented diets compared to the other diets resulted in strengthening the non-specific defense system of shrimp to bacteria and pathogens. Smith et al. (2003) demonstrated that the decrease of immune parameters may be due to the consumption of the hemocytes by degranulation. In this study, zinc sulfate reduced the hemocyte quantity, and decreased the values of some immune parameters tested. This could result in lower survival rate of the shrimps fed zinc sulfate. Moreover, circulating hemocytes are affected by extrinsic factors like temperature, salinity, pH, and heavy metals (Le Moullac and Haffner, 2000). Therefore, different responses in hemocyte count are considered due to the different zinc sources tested. To our knowledge, the mechanism by which organic zinc sources increase numbers of hemocytes is still not elucidated to date.

Zinc is well-known to play a central role in the immune system (Rink and Kirchner, 2000). In the present experiment, AKP, SOD, PA and PO exhibited a higher activity in the zinc-supplemented group, as demonstrated in other reports (Apines et al., 2003; Apines-Amar et al., 2004; Tan and Mai, 2001). Furthermore, ZnMet showed better immune activity compared to the other zinc sources. This implied that ZnMet was the most effective one in enhancing innate immune responses of shrimps. Besides its involvement in genetic stability and gene expression through the activity of transcription factors and RNA and DNA polymerases, Zn also plays a role in DNA repair and apoptosis (Dreosti, 2001; Falchuk, 1998). Apines et al. (2003) and Apines-Amar et al. (2004) reported that the expression of DNA polymerase and CuZnSOD was higher in fish fed the chelate than the inorganic source, which could have enhanced the rate of protein synthesis. This may help explain the enhanced growth of the shrimp in this study.

In addition, levels of zinc also affect animal immunity. Studies demonstrated that the adequate dietary Zn has immunostimulatory activities, and keeps the homeostasis of oxidation/reduction, but excessive dietary Zn induces a high oxidative stress in *P. monodon* (Shiau and Jiang, 2006) and in abalone (Wu et al., 2011). Similarly, the supplemental Zn also resulted in a general disturbance of the immune capacities of hemocytes in in vitro experiments (Mottin et al., 2010). Among the zinc sources studied, organic zinc sources showed better immune activity compared to $ZnSO_4$. Interestingly, zinc supplementation from the chelate at the same levels (36 mg/kg of diet) as the inorganic source significantly increased the level of enzyme transcript, indicating that dietary mineral uptake from the chelate sources improved cellular mineral-dependent functions. Likewise, this corresponds to our previous result (Lin et al., 2011) wherein immune activity of shrimp from the chelate was markedly higher compared to the sulfate even at one-half supplementation level of the inorganic salt. The higher immune parameters in the chelate, in this study compared to the sulfate-fed shrimp indicated a higher utilization of Zn. Combined with these findings, it is implied that dietary Zn could induce expression of antioxidant molecules (e.g., superoxide dismutase and metallothionein) and then maintain a better antioxidant status against oxidative stress in human and animals (Fang et al., 2002).

The present study showed that amino acid-chelated zinc significantly reduced cumulative mortality of *L. vannamei* after being challenged by *V. harveyi*. The improved resistance of *L. vannamei* after challenge may be partly attributable to the increased AKP and SOD activity of shrimp compared to zinc sulfate. Therefore, the findings indicated that the increased resistance to *V. harveyi* of *L. vannamei* was related to the enhanced immune status. More studies are needed to find the reasons for it. To the best of our knowledge, the exact mechanisms for these findings were not clear yet.

In summary, the requirement for Zn depends on its chemical form, as suggested by improved growth and hemocyte functions with the supplementation of amino acid-chelated zinc. Results of this study indicate that the level of Zn recommended by NRC (2011) is sufficient for optimal growth performance and immune responses, and that Zn from organic sources improves these measurements when supplemented to diets containing 30 ppm of supplemental Zn from ZnMet. Thus, based on weight gain, survival and immunity indices examined in this study, amino acid-chelates are a better source of trace elements for *L. vannamei*. Potential benefits of organic zinc sources on hemocyte functions need to be further evaluated.

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