The Efficacy of Mineral-Amino Acid Complex (Zn, Mn, Cu, Fe and Se) in Diets to Growth Performance, Immune Status and Meat Quality of White Shrimp, *Litopenaeus vannamei*

Orapint Jintasataporn^{1*} Terry Ward², Srinoy Chumkam³, and Oratai Jintasataporn⁴

¹Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand
Email: ffisora@ku.ac.th

²Zinpro Corporation, Eden Prairie, MN USA

³Faculty of Agricultural Technology, Valaya Alongkorn Rajabhat, University Under the Royal Patronage, Pathum Thani, Thailand

⁴Department of Animal Science, Faculty of Agriculture at Kamphaeng, Kasetsart University Kamphaeng Campus, Nakornprathom, Thailand

*Correspondence to: ffisora@ku.ac.th

Abstract

Orapint Jintasataporn, Terry Ward, Srinoy Chumkam, and Oratai Jintasataporn. 2015. The Efficacy of Mineral-Amino Acid Complex (Zn,Mn,Cu,Fe and Se) in Diets to Growth Performance, Immune Status and Meat Quality of White Shrimp, *Litopenaeus vannamei*. *Aquacultura Indonesiana*, 16 (1): 33-37. The efficacy of mineral-amino acid complex of Zn, Mn, Cu, Fe and Se for white shrimp, *Litopenaeus vannamei*, diet compare to inorganic mineral on growth performance, feed utilization and immunity was studied. The treatments were 1) the control diet with contained inorganic minerals of Zn, Mn, Cu, Fe and Se (T1-C1X). 2) treatment diet of the combination of inorganic mineral and organic mineral-amino acid complex 70:30 at the same level of control diet Availa Zn, Availa Cu, Availa Mn, Availa-Fe 100 and Availa-Se 1000 (T2- Inorg:org 1X) and 3) The sole mineral-amino acid complex Av complex of aila Zn, Availa Cu, Availa Mn, Availa-Fe 100 and Availa-Se 10000 at 0.5X (T3-Org0.5X). The results indicated that there were not significantly different ($P>0.05$) on the growth performance, feed consumption, feed conversion ratio, Protein efficiency ratio and survival rate. The immune status in term of hemocyte count were significantly increase ($P<0.05$) in group of shrimp fed mineral-amino acid complex than control. Drip loss of peel white shrimp on 96 h was low ($P<0.05$) in group of shrimp fed mineral-amino acid complex than control. Therefore, mineral-amino acid complex of Zn, Mn, Cu, Fe and Se at 0.5X (T3-Org 0.5X) exhibited the same growth performance, feed utilization and survival rate as inorganic mineral (T1-C1X) and combination of inorganic and organic mineral-amino acid complex (T2- Inorg:org 1X). Hence, organic mineral-amino acid complex has the efficacy around 200% of inorganic mineral on shrimp growth performance.

Keywords : Growth performance; Immunity; Meat quality; Mineral-amino acid complex (Availa Zn,Mn,Cu, Fe and Availa Se); White shrimp

Introduction

Nutrition encompasses the chemical and physiological process which provides nutrients to an animal for normal function, increase in immunity, disease resistance, maintenance and growth. It involves ingestion, digestion, absorption and transportation of nutrients and removal of waste (Murry *et al.*, 1993). Minerals are essential elements required for normal growth and are indispensable in the diet (Yamaguchi, 1998). Some minerals are required in considerable quantities and termed macro-elements while others which are required in lesser amounts are referred to as micro-elements (trace minerals). The trace minerals are having greater role in shrimp nutrition along with other minerals for healthy shrimp and better production

(National Research Council;NRC, 2011). Trace minerals such as zinc, copper, manganese, selenium, and iron are essential in aquatic animals for a variety of processes such as skeletal structure, electron transfer, acid-base equilibrium, osmoregulation, enzymatic activities, immune competence and basic metabolic functions. Mineral deficiencies manifested as poor growth, low feed efficiency and high mortality have long been recognized. Generally, aquatic animals can obtain the majority of their essential minerals from the external aquatic environment, there are some minerals whose amounts are limited in the water and need to be provided in the feed (Lall, 1989). Traditionally in the aquatic feed industry those minerals have been provided as salts of inorganic trace minerals. These inorganic salts have the advantage of being comparatively cheap

but suffer from very low levels of availability to the animal. A problem of inorganic mineral in terms of bioavailability especially sulphate form, a frequently used inorganic mineral, comes from the high solubility and readily dissociate in solution as the active trace mineral cations (Zn^{2+} , Cu^{2+} , Mn^{2+} , Fe^{2+} and Se^{2+}) which can interact with dietary components such as tannins and phytic acid to form insoluble complexes. Apart from complex formation highly reactive free metal cations are known to interfere with absorption of each other, an example being the interaction between calcium tri-phosphate, abundant in the fish meal ash, with divalent cations such as Zn and Cu, leading to deficiency symptoms of these minerals. Ashmead (1992) reported that the organic mineral, zinc amino acid complex, has been shown to have a higher absorption rate in animal intestine than inorganic forms of zinc. Hardy and Shearer (1985) found that feeding a zinc-amino acid chelate resulted in greater zinc deposition in body tissues than zinc sulfate or zinc sulfate EDTA in low calcium-phosphorus diets but not in high calcium-phosphorus diets.

The objectives of the study are to evaluate the effect of mineral- amino acid complex of Zn, Mn, Cu, Fe and Se on growth performance, and immunity of white shrimp, *Litopenaeus vannamei*

Materials and methods

Trial design

The experiment was assigned in a completely randomized design (CRD) by varying the proportion of inorganic mineral and organic mineral of mineral-amino acid complex of Zn, Mn, Cu, Fe and Se (Availa-Zn120, Availa-Mn80, Availa-Cu100, Availa-Fe100 and Availa-Se1000 from Zinpro Corporation, USA). The control diet (T1-C1X) was contained inorganic minerals of $ZnSO_4$ 120 mg/L, $MnSO_4$ 60 mg/L, $CuSO_4$ 32 mg/L, $FeSO_4$ 100 mg/L and sodium selenite 0.3 mg/L. The treatment diet (T2- Inorg:org 1X) is the combination of inorganic mineral and organic mineral-amino acid complex at the same level of control diet by using mineral-amino acid complex of Zn, Mn, Cu, Fe and Se at 50, 20, 10, 50, and 0.3 mg/L replace for inorganic mineral and the other treatment (T3-Org 0.5X) diet of the sole mineral-amino acid complex which was 50% of control diet that composed of Zn, Mn, Cu, Fe and Se at 60, 30, 16, 50, and 0.15 mg/L. The study

conducted on 3 treatments with 6 replications per treatment.

Diet Preparation

The test diet was formulated based on the shrimp nutrient requirements (NRC, 2011). Three iso-nitrogenous diets of 35% CP, 7.5% lipid and 1.0% fiber were pelleted for this study. Feed was composed of 25% fishmeal, 20% soybean meal, 5% shrimp and squid meal, 10.9% poultry meal, 26.4% wheat flour and broken rice, 5.4% fish, soya and squid liver oil, 1.1% vitamin premix, 4% Mono-dicalcium phosphate, 1.7% binder and 4.4% mineral premix with different proportion of inorganic and organic mineral. Each treatment was supplemented inorganic and/or organic mineral-amino acid complex at different proportions as designed above.

All materials were grounded and passed through a 250 μm screen then well mixed for 15 min, whereafter oil was gradually added while mixing constantly. Water was slowly added and then entire diet was pelleted bypassing through Hobart mincer. Pellet of 1.8 mm were dried at 65°C and stored in plastic bags at room temperature until fed. The diets were determined feed compositions by proximate analysis follow methods of AOAC (2000)

Experimental conditions

The experiment was carry out in 18 fiber tanks with 1,000 L capacity and filled with 12‰ saline water, DO >5.0 mg/L, pH 7.5-7.8 at the volume of 500 L and stocked juvenile white shrimp of 4.43 g at a density of 70 shrimp/m². Feed was applied to shrimp 4 times a day at 2.5-3% of body weight for 8 wk.

Data collection

At the end of 8 wk, growth performance was recorded on live weight gain, specific growth rate, survival rate, feed conversion ratio (feed consumption/fish production) and protein efficiency ratio.

The immunity in terms of total hemocyte count, total protein (Lowry *et al.*, 1951) and prophenol oxidase activity were determined follow method of Encarnacion *et al.* (2012).

Meat quality was evaluated by sample the peel white shrimp meat and then chilled for 96 h. Drip loss was determined follow method of AOAC (2000) by weight sample before chilled at 4°C and after chilled for 96 h. The value of difference in percentage was drip loss.

Statistical analysis

All data was analyzed by one-way ANOVA (analysis of variance). The Duncan's Multiple Range Test was used to determine the differences between the treatment means. The alphabetical notation was used to mark the differences at significant level of an alpha 0.05. All research was conducted at the Nutrition and Aquafeed Laboratory, Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand.

Results

The efficacy of inorganic and organic mineral at the proportion of inorganic mineral and mineral-amino acid complex (Availa-Zn120, Availa-Mn80, Availa-Cu100, Availa-Fe100 and Availa-Se1000) on growth performance, immune status and meat quality was conducted. The results as following:

Growth performance

The effect of inorganic and organic mineral-amino acid complex on growth performance and feed utilization was presented in Table 1. The result showed that there were not significantly different ($P>0.05$) on the growth performance, feed utilization and survival rate. The relative growth rate (percentage of weight increased compare with control group) of shrimp fed diet composed of combination of inorganic and organic mineral-amino acid complex (T2-Inorg:org 1X70:30) and diet composed of the sole organic mineral-amino acid complex (T3-Org 0.5X) were increased 5.6-9.6% when compare to control (T1-C1X) group of inorganic mineral but there were not significantly different ($P>0.05$). Although feed contained 50% of normal level (T3-org 0.5X) still exhibited the same growth performance as control (T1-C1X). These implied that organic mineral-amino acid complex has the efficacy 200% of inorganic mineral on shrimp growth performance.

Table 1. Growth performance and feed utilization of white shrimp fed diet composed of inorganic and organic mineral-amino acid complex for 8 wk (means $\pm$ SD)

Parameter	T1-C1X Inorganic	T2- Inorg:org 1X 70:30	T3-Org 0.5X	P-value
Initial production (g/tank)	264.47 ^a $\pm$ 13.13	267.23 ^a $\pm$ 14.97	265.93 ^a $\pm$ 12.52	0.9398
Final production (g/tank)	542.79 ^a $\pm$ 83.51	601.09 ^a $\pm$ 47.11	624.35 ^a $\pm$ 44.99	0.0903
Initial weight (g/ind.)	4.41 ^a $\pm$ 0.22	4.45 ^a $\pm$ 0.25	4.43 ^a $\pm$ 0.21	0.9428
Weight at 8 wk (g/ind.)	11.12 ^a $\pm$ 1.14	11.54 ^a $\pm$ 0.75	11.79 ^a $\pm$ 0.71	0.4387
Weight gain at 8 wk (g/ind.)	6.71 ^a $\pm$ 1.15	7.09 ^a $\pm$ 0.52	7.35 ^a $\pm$ 0.69	0.4192
Average daily gain at 8 wk (g/ind./d)	0.12 ^a $\pm$ 0.02	0.13 ^a $\pm$ 0.01	0.13 ^a $\pm$ 0.01	0.3268
Relative growth (%)	0.00 ^a $\pm$ 17.15	5.64 ^a $\pm$ 7.70	9.63 ^a $\pm$ 10.22	0.4202
Specific growth rate at 8 wk (g/ind.)	1.51 ^a $\pm$ 0.19	1.58 ^a $\pm$ 0.12	1.62 ^a $\pm$ 0.11	0.4122
Accumulate feed consume at 8 wk (g/ind.)	9.52 ^a $\pm$ 1.04	9.33 ^a $\pm$ 0.82	9.60 ^a $\pm$ 0.40	0.8315
Daily feed consume at 8 wk (g/ind.)	0.17 ^a $\pm$ 0.02	0.17 ^a $\pm$ 0.01	0.17 ^a $\pm$ 0.01	0.7613
Feed conversion ratio at 8 wk	1.44 ^a $\pm$ 0.20	1.32 ^a $\pm$ 0.11	1.32 ^a $\pm$ 0.16	0.3355
Survival rate at 8 wk (%)	81.39 ^a $\pm$ 9.33	86.94 ^a $\pm$ 6.36	88.33 ^a $\pm$ 4.59	0.2286
Protein efficiency ratio	1.99 ^a $\pm$ 0.29	2.14 ^a $\pm$ 0.18	2.15 ^a $\pm$ 0.26	0.4899

Means within the same row with different superscripts differ ($P\leq 0.05$)

Table 2. Immune response of white shrimp after fed diet composed of inorganic and organic mineral-amino acid complex for 8 wk (means $\pm$ SD)

Parameter	T1-C1X Inorganic	T2- Inorg:org 1X 70:30	T3-Org 0.5X	P-value
Hepatosomatic index (%)	3.45 ^a $\pm$ 1.05	4.19 ^a $\pm$ 1.07	3.86 ^a $\pm$ 0.56	0.3998
Total hemocyte count ($\times 10^7$ cell/mL)	2.90 ^b $\pm$ 0.17	4.18 ^a $\pm$ 1.04	3.36 ^{ab} $\pm$ 0.81	0.0323
Total Protein (mg/mL)	6.48 ^a $\pm$ 0.90	6.40 ^a $\pm$ 1.59	5.16 ^a $\pm$ 0.44	0.1006
Phenol oxidase activity (unit/min/mgprotein)	161.79 ^a $\pm$ 47.36	178.49 ^a $\pm$ 13.71	198.07 ^a $\pm$ 37.40	0.2444

Mean values within the same row with different superscripts were significantly different ($P\leq 0.05$)

Table 3. Shrimp meat quality after harvested of white shrimp fed diet composed of inorganic and organic mineral-amino acid complex for 8 wk (means $\pm$ SD)

Items	T1-C1X Inorganic	T2- Inorg:org 1X 70:30	T3-Org 0.5X	P-value
Head (%)	35.37 ^a $\pm$ 3.60	36.76 ^a $\pm$ 2.02	37.36 ^a $\pm$ 1.32	0.8984
Tail (%)	64.63 ^a $\pm$ 3.60	63.24 ^a $\pm$ 2.02	62.64 ^a $\pm$ 1.32	0.8984
Peel shrimp chilled for 96 h				
Drip loss (%)	2.92 ^b $\pm$ 0.37	2.25 ^a $\pm$ 0.23	2.03 ^a $\pm$ 0.09	0.0130

Mean values within the same row with different superscripts were significantly different ($P \leq 0.05$)

Immune status

The immune status in term of prophenol oxidase activity, total protein and hepatosomatic index in Table 2 showed no significantly difference ($P > 0.05$). Hemocyte count in group of combination mineral of T2-Inorg:org 1X 70:30 were $4.18 \pm 1.04 \times 10^7$ cell/mL, group of the sole organic mineral-amino acid complex of T3-Org 0.5X were $3.36 \pm 0.81 \times 10^7$ cell/mL which significantly higher ($P < 0.05$) than inorganic mineral of T1-C1X, $2.90 \pm 0.17 \times 10^7$ cell/mL.

Meat quality

Shrimp meat quality after harvest was determined. Head-tail ratio by percentage of head and tail showed no significantly different ($P > 0.05$). Drip loss after chilled for 96 h showed significantly different ($P < 0.05$). The drip loss in organic mineral-amino acid complex of T3-Org 0.5X group was lower ($P < 0.05$) than combination of inorganic and organic mineral-amino acid complex of T2-Ing:org 1X 70:30 and inorganic mineral of T1-C1X (Table 3).

Discussion

The efficacy of mineral-amino acid complex (Zn, Mn, Cu, Fe and Se) on white shrimp, *Litopenaeus vannamei*, diets perform in the same trended as the research on chelated copper in white shrimp fed diet containing phytic acid that reported shrimp required 3–4 times more dietary copper from copper sulfate than copper from a chelated copper source to promote comparable growth (Bharadwaj *et al.*, 2014). The accumulation or bioavailability of organic mineral is better than inorganic mineral such as a zinc-amino acid complex or zinc amino acid chelate resulted in better growth performance and greater zinc deposition in body tissues than zinc sulfate (Lin *et al.*, 2013). Not only the form of mineral but the macro mineral like calcium and phosphorus in diet also exhibit some antagonism on inorganic mineral accumulation. Feeding

a zinc-amino acid chelate results in greater zinc deposition in body tissues than zinc sulfate or zinc sulfate EDTA in low calcium-phosphorus diets but not in high calcium-phosphorus diets (Hardy and Shearer, 1985). Hence, the growth performance may related to the mineral or salinity in water culture media. Therefore, the sole organic mineral-amino acid complex (T3-Org 0.5X) has the efficacy 200% of inorganic mineral by exhibited the same growth performance, feed utilization and survival rate as inorganic mineral (T1-C1X) and combination of inorganic and organic mineral-amino acid complex of 1X at 70:30 (T2- Inorg:org 1X). The healthy shrimp has high immunity especially the high number of hemocyte count and hemocyte activity in term of phenol oxidase activity which is the oxidizing agent for protect animal from pathogen infection. The organic mineral-amino acid complex in both T2-Inorg:org 1X 70:30 and T3-Org 0.5X can increase amount of hemocyte cell which is the cell in defense system of shrimp. Many researches on mineral-amino acid complex and chelated mineral had performance in the same trended (Lin *et al.*, 2013; Bharadwaj *et al.*, 2014). The high levels of mineral-amino acid complex of Zn, Mn, Cu, Fe and Se in white shrimp meat decrease drip loss which accelerates the shelf life. The improving of white shrimp meat quality related to the high efficacy of mineral-amino acid complex to promote the firmness of muscle for holding the water in the tissue. The high bioavailability, absorption, utilization and accumulation of Zn, Mn, Cu, Fe and Se in white shrimp involve in protein synthesis, carbohydrate utilization, shell formation, non-specific immune system and enhance oxidative scavenging enzyme like superoxide dismutase, peroxidase, glutathione in cell which promote muscle yield and meat quality.

Conclusion

The mineral-amino acid complex at 0.5X (T3-Org 0.5X) of Zn, Mn, Cu, Fe and Se: Availa-

Zn 60 mg/L, Availa-Mn 30 mg/L, Availa-Cu 16 mg/L, Availa-Fe 50 mg/L and Availa-Se 0.3 mg/L (Zinpro Corporation, USA) exhibited the same growth performance, feed utilization and survival rate as inorganic mineral (T1-C1X) which composed of ZnSO₄ 120 mg/L, MnSO₄ 60 mg/L, CuSO₄ 32 mg/L, Fe 100 mg/L and sodium selenite 0.3 mg/L and combination of inorganic and organic mineral-amino acid complex of 70:30 (T2 Ing:org 1X 70:30) which using mineral-amino acid complex of Zn, Mn, Cu, Fe and Se : Availa-Zn, Availa-Mn, Availa-Cu, Availa-Fe and Availa-Se 50, 20, 10, 50 and 0.3 mg/L replaced for inorganic mineral. The mineral-amino acid complex of Zn, Mn, Cu, Fe and Se (T2 Ing:org 1X 70:30 and T3-Org 0.5X) can enhance the non-specific immune system of white shrimp and improve white shrimp meat quality by reducing drip loss in peel white shrimp meat after chilled for 96 h. Hence, organic mineral-amino acid complex has the efficacy around 200% of inorganic mineral on white shrimp growth performance.

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