Effect of Trace Minerals Concentration on Growth Performance and Immune Response of White Shrimp (*Litopenaeus vannamei*)

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Abstract

Trace minerals are important because they act as cofactors of a variety of enzymes. They involved improving growth performance, metabolism and immune system of shrimp especially in case of high plant basal feed. Since the insufficient amount of fish meal production due to unsustainable fishery problems, soybean meal has been a major alternative protein source but phytic acid in the plant was the factor to reduced bioavailable of minerals. So, this research studied the effect of trace mineral concentration in the diets on growth performance and immune response of white shrimps *Litopenaeus vannamei.* The trial was assigned in CRD with 3 treatments and each of 10 replicates. The diets with 37% crude protein and 5% crude fat were formulated and top up with different trace mineral levels of 1x, 2x and 3x. All diets were fed to shrimps for 28 days and the results showed no significant differences ($P>0.05$) on all growth performance parameters. Anyway, the numerical values of growth performance had an improving trend when increasing mineral concentrations. Furthermore, an immune response such as total haemocyte count, superoxide dismutase inhibition and glutathione showed no significant difference between treatments ($P>0.05$) but phenoloxidase activity showed increasing values when minerals concentration had a higher dose and significant difference among the group ($P<0.05$). Therefore, the results of this study clearly showed an increasing dose of trace minerals in the white shrimp diet trend to promote growth performance and could improve immune response especially phenoloxidase activity.

Keywords: trace minerals supplementation, high soybean meal, *Litopenaeus vannamei*, growth performance, immune response

Introduction

Fish meal is a raw material that is a source of protein that has a large amount of use in the aquaculture industry due to the high protein and balancing amino acids (Tacon & Metian, 2008). But since the fishing industry currently faces various problems (Naylor et al. 2009), resulting in the reduction of fishmeal production around the world and the trend of continuous decline. Therefore, feed factories around the world need to improve feed formulas in response to such changes. White shrimp (*Litopenaeus vannamei*) are very important aquatic animals for the aquatic industry because it is an animal that has been raised and exported in the top rank of the world. White shrimp is an animal that can eat both animal and plant sources (Omnivorous). They have the ability to digest and absorb nutrients well even if using any raw materials in any recipe.

From the situation of world fishmeal production declining, the price is constantly rising together with improper use of labor and illegal fishing problems, resulting in adaptation of the animal feed industry around the world such as reducing the amount of fishmeal in animal feed diets and recruiting alternative protein raw materials to substitute fishmeal. Many protein materials are used, animal protein raw materials such as chicken meal, meat and bone meal, fish head meal, squid meal, squid liver powder, blood powder meal, chicken feather powder, or plant protein ingredients such as soybean meal, soy protein concentrated, rapeseed meal, canola meal, etc. Especially soybean product which have high production capacity and cheaper price. Therefore, soybean product has been used in animal feed formulas for a long time and no adverse effects when used in quantities appropriate to the type of each animal. Nevertheless, high usage of soybean meal in shrimp diets could reduce the bioavailability of protein and trace minerals such as copper (Cu), zinc (Zn), manganese (Mn) and selenium (Se) because the phytic acid in soybean meal binds trace minerals so animal cannot absorb minerals and excrete them to environment.

Trace minerals are important modules of enzymes because they act as cofactors and activators of a variety of enzymes and they involved in improved growth performance, metabolism and immune system of shrimp especially in case of high plant basal feed. Therefore, this research focus on the supplementation of certain minerals (Cu, Zn, Mn, and Se) in white shrimp diet recipes that use high soybean meal to enhance the growth of the white shrimp (*Litopeneaus vannamei*).

Objectives

To study the effect of trace mineral supplementation in white shrimp diet with soybean meal 40% on growth performance and immune response of white shrimp.

Methodology and data collection

1. Experimental diets and Culture conditions

Three diets were formulated to contain 370 g/kg crude protein, 50 g/kg crude lipid and 70 g/kg ash (Table 2). Three practical diets with graded levels of trace minerals (1x, 2x, and 3x) were formulated. The raw materials were ground and then passed through a 500-micron mesh. After that, all raw materials were mixed by a mixer machine and the dough was pelleted through a 2 mm die, using extrusion machinery. The pellets were steamed at 100 degrees Celsius for 6 minutes and dried at 90 degrees Celsius in an oven for 6 hours to a moisture content of 9% - 10%. Lastly, the diets were sealed in plastic bags stored at room temperature until used. Proximate composition analysis and mineral profiles of diets are given in Table 2 and analyzed by AOAC (2000) method. The experiment proceeded at the private farm (Samut Songkhram province, Thailand). White shrimp, *Litopenaeus vannamei*, was obtained from commercial hatcheries (Samut Songkhram province, Thailand). The 1,800 healthy shrimp (*Litopenaeus vannamei*) with an average weight of 0.6997 ± 0.0039 grams were selected and randomly transferred into 30 cages (1x1x1.2 m cages), 10 replicate per treatment at a density 60 shrimp per cage, which are paved with polyethylene whole ponds. All shrimp were fed by auto-feeder five times per day at 8:00, 10:00, 14:00, 16:00 and 20:30. During the trial period, water quality parameters were monitored and maintained at salinity 15 ppt, dissolved oxygen was higher than 5 mg/L, pH 7.5-8.5, water temperature ranged from 28 to 30 degrees Celsius and nitrite was lower than 0.5 mg/L throughout the culture period for 4 weeks.

Table 1: Experimental feed formula (%)

Ingredients	1x	2x	3x
Fish meal	15.00	15.00	15.00
Animal protein	5.50	5.50	5.50
Soybean meal	40.00	40.00	40.00
Wheat flour	34.13	34.07	34.01
Fish oil	2.00	2.00	2.00
Lecithin	0.50	0.50	0.50
Methionine	0.05	0.05	0.05
Multivitamin	1.30	1.30	1.30
Calcium carbonate	0.70	0.70	0.70
Magnesium sulfate	0.30	0.30	0.30
Potassium chloride	0.30	0.30	0.30
Iron amino acid complex	0.10	0.10	0.10
Organo Mix-P	0.12	0.18	0.24

Remark: Organo Mix-P is the commercial mix of trace minerals including zinc, manganese, copper, and selenium.

Table 2: Proximate composition of experimental feed (% dry matter)

Composition	1x	2x	3x
Moisture (%)	10.1	10.7	9.98
Crude protein (%)	37.6	37.2	37.8
Crude lipid (%)	4.46	5.16	5.15
Ash (%)	7.80	7.70	7.79
Calcium (%)	1.54	1.57	1.55
Phosphorus (%)	0.979	0.995	0.976
Zinc (mg/kg)	135	179	222
Manganese (mg/kg)	86.7	136	174
Copper (mg/kg)	17.5	22.2	27.4
Selenium (mg/kg)	2.08	2.26	2.64

2. Sample collection

2.1 Growth performance and feed utilization

At day 28th of culture, the final body weight and total production of white shrimp was recorded then data were calculated such as percent weight gain (WG), average daily growth (ADG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER). The parameters were calculated as follows:

A. Percent weight gain (WG, %) = 100 × (Wt –Wi) / Wi

B. Average daily growth (ADG, g day-1) = Wt –Wi / day

C. Specific growth rate (SGR, % day-1) = 100 × (LnWt – LnWi) / t,

D. Feed conversion ratio (FCR) = feed consumed (g) / weight gain (g)

E. Protein efficiency ratio (PER) = weight gain (g, wet weight) / protein intake (g, dry weight)

Where Wt is the final body weight (g), Wi is the initial body weight (g), and t is the experimental duration in days.

2.2 Immune response

At day 28th of culture, the hemolymph of six shrimp per cage was randomly collected to determine total haemocyte count, phenoloxidase, superoxide dismutase, glutathione by Test Kit.

3. Statistical analysis

The results were presented as means ± standard deviation. Completely randomized design (CRD) was assigned and all data were analyzed by one-way ANOVA (analysis of variance). Duncan's procedure was used for multiple comparisons on the differences between the treatment means. Differences were regarded as significant when $P<0.05$. The alphabetical notation was used to mark the differences at a significant level of an alpha 0.05.

Results and Discussion

1. Growth performance and feed utilization

The growth performance and feed utilization of the white shrimps fed different diets were showed in Table 3. Final body weight ranged between 6.00 ± 0.40 g to 6.39 ± 0.34 g, total production ranged between 317.8 ± 23.9 g to 341.5 ± 20.7 g, weight gain ranged between 659.7 ± 57.2 g to 716.3 ± 49.6 g, average daily growth ranged between 0.18 ± 0.01 g/shrimp/day to 0.19 ± 0.02 g/shrimp/day, specific growth rate ranged between 7.15 ± 0.22 to 7.37 ± 0.18, feed conversion ratio ranged between 0.87 ± 0.06 to 0.95 ± 0.08, and protein

efficiency ratio ranged from 2.82±0.25 to 3.06±0.22. There were no significant differences among the groups (*P*>0.05).

Table 3: Growth performance and feed utilization of white shrimp (*Litopenaeus vannamei*) fed vary trace minerals concentration feed for 28 days (mean±SD)

Parameters	1x	2x	3x	*P*-value
FBW (g/shrimp)	6.00 ± 0.40	6.26 ± 0.67	6.39 ± 0.34	0.241
TP (g)	317.8 ± 23.9	333.7 ± 40.0	341.5 ± 20.7	0.241
WG (%)	659.7 ± 57.2	697.8 ± 95.7	716.3 ± 49.6	0.241
ADG (g/shrimp/day)	0.18 ± 0.01	0.19 ± 0.02	0.19 ± 0.01	0.240
SGR (%/day)	7.15 ± 0.22	7.29 ± 0.35	7.37 ± 0.18	0.231
FCR	0.95 ± 0.08	0.90 ± 0.11	0.87 ± 0.06	0.211
PER	2.82 ± 0.25	3.00 ± 0.40	3.06 ± 0.22	0.258

Remark: Data without superscript letters in the same row indicates no significantly difference (*P*>0.05). FBW = Final body weight, TP = Total production, WG = Weight gain, ADG = Average day growth, SGR = Specific growth rate, FCR = Feed conversion ratio, PER = Protein efficiency ratio.

In this experiment, 40 percent soybean meal was used to be the main protein source in *Litopenaeus vannamei* shrimp feed and the growth performance of this experiment showed that all parameters of the shrimp fed with 3 different trace minerals level (1x, 2x, 3x) were not significantly different (*P*>0.05) although 40 percent soybean meal was used as the main source of protein in feed formulations. Based on research from Ghorbani et al. (2017) found that soybean meal 42 percent in the white shrimp diets didn't negatively affect to final weight, final production, feed conversion ratio, protein efficiency ratio, survival rate and no significant difference from shrimp fed commercial feed with 39 percent crude protein (*P*>0.05). Moreover, the research of Katya et al. (2016) showed the final body weight and weight gain of *Litopenaeus vannamei* shrimp fed basal control were significantly lower than the shrimp fed 0.5% trace mineral (Cu, Mn, and Zn) premix diets. The first mineral is copper, Cu is an essential element for all organisms. Second is manganese, its functions as a cofactor in several enzyme systems, including glucose oxidation, amino acid metabolism and fatty acid metabolism (Lall, 2002). Next is zinc, roles as a cofactor and a component of metalloenzymes such as alkaline phosphatase and DNA polymerases (NRC, 2011). According to Lin et al. (2013), zinc effects on growth and survival of *Litopenaeus vannamei* shrimp especially organic zinc (ZnMet) that showed the highest weight gain parameters when compared with no adding zinc and add

other forms. Huang et al., (2017), which supplemented and adjusted the mineral balance of recipes using soybean meal, 46.6 percent was the main protein source close to the formula used as fishmeal as the main protein source. It was found that growth rates, survival rates, levels of copper, manganese and zinc accumulation in the shrimp body of the formula that used soybean meal as the main protein source were not significantly different from the formulas that used the fishmeal as the main source of raw protein.

2. Immune response

The immune response of the shrimp fed different diets were shown in Table 4. The Total haemocyte count ranged from $11.0x10^4$±0.50 to $14.2x10^4$±4.80 cell/ml. Phenoloxidase enzyme of treatment 3x showed the highest value of 103.02±14.73 unit/min and significant difference from other groups (P<0.05).

Table 4: Immune response of white shrimp (*Litopenaeus vannamei*) fed for 28 days (mean±SD)

Parameters	1x	2x	3x	P-value
THC ($x10^4$ cell/ml)	14.2±4.80	11.0±0.50	12.7±2.25	0.494
PO (unit/min)	$68.2±6.64^a$	$81.8±2.45^a$	$103.02±14.73^b$	0.011
SOD (inhibition rate %)	90.33±0.06	90.20±0.36	90.17±0.15	0.659
Glutathione (nM)	0.55±0.09	0.55± 0.05	0.35± 0.26	0.280

Note: Data without superscript letters in the same row indicates no significantly difference (P>0.05). THC = Total haemocyte count, PO = phenoloxidase, SOD = Superoxide dismutase inhibition.

This research found that phenoloxidase activity (PO) of treatment 3x showed the highest values of 103.02±14.73 units/min and significant difference when compared to the treatment of 1x and 2x (P<0.05). Lin (2013) demonstrate that zinc 30 ppm could enhance PO activity and significant difference from control (P<0.05). Activities of the two major antioxidant enzymes SOD and glutathione responsible for combating the effects of ROS vary widely in relation to many factors. Selenium is capable of generating glutathione because it is an important component of glutathione peroxidase, which is characterized as a tetrameric protein with four atoms of Se per molecule (Rotruck et al., 1973). Wang (2006) showed that higher Se intakes did not influence the generation of oxidative stress such as SOD and glutathione in *Litopenaeus vannamei* as same as the result of this experiment, glutathione activity showed no significant difference (P>0.05).

According to many researchers, they were shown that the used of legumes together with the used of other animal protein raw materials can effectively replace the used of fish meal in white shrimp *Litopenaeus vannamei* diets, without any negative impact on the growth performance. In this study, the shrimp were fed by 2x and 3x numerical value to show increasing numerical trend such as final weight, average daily gain, and specific growth rate when compared to the 1x group even though there did not significant differences ($P>0.05$). Therefore, the properties of trace minerals were also considered when used high protein plant materials in feed diets to improve the growth performance of shrimp.

Conclusion

This experiment showed that increasing doses of trace minerals in the white shrimp (*Litopenaeus vannamei*) feed presented the numerical trend to improve growth performance and could improve immune response. This is because trace minerals are an important component of an enzyme in metabolism, growth/sex hormone, and DNA/RNA replication.

Suggestion/Further Study

1. To study the effect of trace minerals supplementation in other species.

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