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Efficacy of inorganic and chelated trace minerals (Cu, Zn and Mn) premix sources in Pacific white shrimp, *Litopenaeus vannamei* (Boone) fed plant protein based diets

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ABSTRACT

The present experiment was conducted to evaluate the efficacy of trace minerals (Cu, Zn and Mn) premixes from inorganic and chelated (chelated to 2-hydroxy-4-methylthiobutanoic acid or hydroxy analog of methionine; Mintrex™) sources, in juvenile Pacific white shrimp, (*Litopenaeus vannamei*) fed plant protein based practical diets. Eight experimental diets comprising a trace minerals (Cu, Zn and Mn) deficient Basal control, and diets supplemented with the trace mineral premixes at four different levels of 2.5 ($M_{2.5}$), 5 (M_5), 7.5 ($M_{7.5}$) and 8.5 g/kg ($M_{8.5}$) from chelated source and at three different levels of 5 (I_5), 8.5 ($I_{8.5}$) and 20 g/kg (I_{20}) from inorganic source were formulated. Eleven numbers of juvenile shrimp averaging 0.6 ± 0.01 g (mean ± SD) were fed one of the eight experimental diets in triplicate groups for 8 weeks. At the end of the feeding trial, shrimp fed $M_{2.5}$ and $I_{8.5}$ diets exhibited the similar final weight (FW) and weight gain (WG) ($P < 0.05$). Performance for Cu and Zn content in the hepatopancreas tissues and whole body showed equal efficiency of M_5 compared to $I_{8.5}$ diet ($P < 0.05$). Whereas, nonspecific enzyme, Cu—Zn super oxide dismutase (Cu—Zn SOD) from the serum and hepatopancreas tissue was recorded to be peaked for the group of shrimp fed M_5 diet. Moreover, plasma protein and glucose levels were recorded to be similar between the groups of shrimp fed $M_{2.5}$ and I_{20} diets ($P < 0.05$). Therefore, the present experiment demonstrated, a higher efficiency of chelated over inorganic source of trace mineral premix in Pacific white shrimp, (*L. vannamei*) fed plant protein based diets.

Statement of relevance

The significance of trace mineral bioavailability has become more important as the composition of majority of commercial fish feed has been changing and there is an increased use of dietary plant protein. As a result, the bioavailability of trace minerals is being adversely affected by the presence of antagonistic factor such as phytic acid in plant protein. Even though, our knowledge in fish nutrition has advanced significantly, the information on trace minerals requirement is still limited and fragmentary. Inorganic form (sulfate/nitrate) of trace mineral has traditionally been used in aquafeed formulation. However, the limited bioavailability of inorganic source of trace mineral due to its higher affinity to antinutrients has hastened the search for alternative form of inorganic trace minerals. Overall performance observed in the present experiments vouched the potential benefit of using chelated trace mineral, Cu, Zn & Mn premix in marine shrimp, Pacific white shrimp to promote the optimum growth, trace minerals saturation in shrimp body and tissue as well as to ensure the optimum enzyme activity and health of cultured shrimp. The present experiment opens a new avenue to compare the efficacy of inorganic and chelated source of trace minerals in other commercially important marine and fresh water fish species. Whereas, the sustainability issue has put a new dimension in aquafeed formulation with a wide array of new ingredients and additives, on the other hand, the importance of basic nutrient such as trace minerals is still in sideline. Substantial investment and integrated scientific efforts are warranted to bridge the knowledge gap and further improve our understandings on the significance of dietary trace mineral in fish nutrition and health at the least cost to environment.

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1. Introduction

The limited availability and rising costs of dietary fish meal have resulted in the increasing use of plant proteins and other animal proteins in shrimp and fish feeds (Naylor et al., 2009; Tacon and Metian, 2008). However, the use of alternate protein sources in feeds may result in the reduced availability of trace minerals such as copper (Cu), zinc (Zn) and manganese (Mn) due to the presence of antagonists such as phytic acid in plant meals and high levels of calcium and phosphorus in animal meals. Phytic acid binds with divalent cationic trace minerals rendering them unavailable to the animal and these are consequently lost to the environment as waste (Cheryan, 1980; Davis and Gatlin, 1996; Davis et al., 1993b; Li and Robinson, 1997). The reduced availability and potential dietary deficiency of trace minerals are of serious concern as Cu, Zn and Mn are the vital trace element required for the normal growth, metabolism and health of aquaculture species including shrimp (Bharadwaj et al., 2014).

Trace minerals are important components of hormones and enzymes serve as cofactors and/or activators of a variety of enzymes as well as participate in a wide variety of biochemical processes (NRC, 2011). Copper is an essential element for all organisms including fish (Watanabe et al., 1997; Lorentzen et al., 1998). It functions in hematopoiesis and in numerous copper dependent enzymes including lysyl oxidase, cytochrome c oxidase, ferroxidase, tyrosinase (O'Dell, 1976). Whereas, Zn is required by fish for many important biochemical processes (Lall, 1989), including growth, protein metabolism, energy production, gene regulation, and maintaining the health of cell membranes and bones (Watanabe et al., 1997). Another important trace mineral, Mn functions as a cofactor in several enzyme systems, including those involved in urea synthesis from ammonia, amino acid metabolism, fatty acid metabolism, and glucose oxidation (Lall, 2002). Thus, ensuring an adequate dietary supply of minerals to farmed fish is essential for proper somatic and skeletal growth, health and final flesh quality (Prabhu et al., 2014).

One of the factors that affect mineral absorption and utilization is their chemical form. Traditionally, supplementation of trace elements to animal diets has been achieved through the use of inorganic salts, such as sulfate and carbonate. However, because of low mineral availability from this source, continuous efforts have been made to improve its utilization both by humans and animals (Apines-Amar et al., 2004a,b). Organic/chelated trace minerals have been viewed as an alternative to inorganic trace minerals. There have been numerous studies in fish and shrimp demonstrating higher availability of trace minerals from chelated sources (Apines et al., 2003; Apines-Amar et al., 2004a,b; Bharadwaj et al., 2014; Buentello et al., 2009; Hardy and Shearer, 1985; Lin et al., 2013; Paripatananont and Lovell, 1995a, 1995b, 1997; Shao et al., 2010). Typically, organic trace minerals are more stable in the digestive tract and less prone to interactions and antagonisms as they are bound to organic molecules and less available to interaction and binding (Bharadwaj et al., 2014). Recently, two reports have been published demonstrating the higher bioavailability of dietary chelated Cu (Bharadwaj et al., 2014) and chelated Zn (Lin et al., 2013) in Pacific white shrimp. There are also reports available on the potential benefit of chelated premix (Cu, Zn and Mn) in rainbow trout, *Oncorhynchus mykiss* (Apines et al., 2003; Apines-Amar et al., 2004a,b). However, limited information is available on the efficacy of dietary trace minerals premixes from the inorganic and/or chelated sources in marine shrimp. Therefore, the present experiment was designed to evaluate the effects of different levels of traditional inorganic and commercial chelated (Mintrex™) sources of dietary trace minerals (Cu, Zn and Mn) premixes on the growth performance, tissue mineralization, serological characteristics and enzyme activity in Pacific white shrimp, *Litopeneaus vannamei* fed plant protein based diets.

Table 1
Composition of the basal diet.

Ingredients	% in diet
Soybean meal	45.00
Wheat flour	27.40
Squid Liver powder	15.00
Cholesterol	0.20
Lecithin	4.00
Fish oil	2.00
Vitamin premix[2]	2.00
Magnesium Oxide[1]	0.35
Potassium Chloride[1]	0.75
Potassium Phosphate[1]	0.50
Cellulose[1]	2.00
MeraMet[3]	0.80
Inorganic mineral[4]	0.00
Mintrex mineral[4]	0.00

[1] Sigma-Aldrich Korea Yongin, Korea.
[2] Vitamin mixtures (contains as mg/kg diet): DL-alpha tocopherol acetate. 60 IU; DL-cholecalciferol, 3000 IU; thiamin, 15 mg; ribo-flavin, 30 mg; pyridoxine, 15 mg; B12, 0.05 mg; nicotinic acid, 175 mg; folic acid, 5 mg; ascorbic acid, 500 mg; inositol, 1000 mg; biotin, 2.5 mg; calcium pantothenate, 50 mg; choline chloride, 2000 mg.
[3] Supplied by Novus International Inc., Canada.
[4] Supplied by Novus International, St. Charles, USA.

2. Materials and methods

2.1. Diets formulation and preparation

Table 1 shows formulation of the Basal diet used in the experiment. Eight isonitrogenous and isocaloric practical diets were formulated to contain minimum level of indigenous trace minerals derived from the ingredients. One of the eight diets comprising a deficient Basal control, and diets supplemented with the trace mineral premixes at four different levels of 2.5 ($M_{2.5}$), 5 (M_5), 7.5 ($M_{7.5}$) and 8.5 g/kg ($M_{8.5}$) from chelated source and at three different levels of 5 (I_5), 8.5 ($I_{8.5}$) and 20 g/kg (I_{20}) from the inorganic source. Mineral premix at 5 g/kg of dietary inclusion were designed to supply the trace minerals, Zn at 65, Cu at 50 and Mn at 40 ppm in the final diet, as per the requirement level of marine shrimp (NRC, 2011). The chelated (MINTREX™ is a trademark of Novus International Inc.) and inorganic trace mineral premix were supplied by Novus International Inc. St. Luis, USA. The trace mineral premix was supplemented at the expense of cellulose in the experimental diets. To match up the antagonism with the commercial practical shrimp diets, high level of dietary soybean at 45% of the diet was added in each experimental diet. Soybean meal (United States Biochemical, Cleveland, OH, USA), fish oil (DHA + EPA enriched; refined fish oil, E-Wha oil Co. Ltd., Pusan, Korea), and wheat flour (United States Biochemical, Cleveland, OH, USA) were used as the main dietary protein, lipid and carbohydrate sources, respectively. All dry ingredients were finely ground, weighed, mixed manually for 5 min and then transferred to a mixer for another 15-minute mixing. Fish oil was then added slowly while mixing was continued. All ingredients were mixed for another

Table 2
Analyzed minerals contents (ppm; DM) of different experimental diets[1].

Diets[2]	Cu	Zn	Mn
Basal	12.8	29.3	25.9
I_5	63.3	90.0	56.0
$I_{8.5}$	99.9	136	85.3
I_{20}	185	182	132.5
$M_{2.5}$	39.3	53.4	43.6
M_5	64.0	93.8	58.8
$M_{7.5}$	86.2	135	73.7
$M_{8.5}$	102	138	87.8

[1] Values are average of duplicates groups of samples.
[2] I: Diets supplemented with Inorganic premix; M: Diets supplemented with Mintrex premix.