Availability of dietary zinc sources and effects on performance of pacific white shrimp *Litopenaeus vannamei* (Boone)

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ABSTRACT

A study was conducted to evaluate the response of Pacific white shrimp *Litopenaeus vannamei* to inorganic or chelated sources of dietary zinc. Two sets of diets, one supplemented with zinc from zinc sulfate (55, 80, 116, 168, 243 and 363 ppm zinc) and the other with zinc from a chelated source (methionine hydroxy analog chelate; 39, 52, 65, 78 and 104 ppm zinc) were fed to replicate groups of juvenile shrimp ($N = 8$; 0.4 g initial weight) for 6 wk. All experimental diets contained 1.38% phytic acid reflecting levels in typical commercial feeds. Final weight, growth rate and biomass of shrimp fed zinc sulfate supplemented diets (243 and 363 ppm total zinc) were significantly higher ($p < 0.05$) than that in shrimp fed the base diet. In contrast, performance of shrimp fed the chelated source of zinc was significantly higher than shrimp in the control group at much lower levels of supplementation (65 and 78 ppm total zinc). Results indicate that shrimp required 3-4 times more dietary zinc from zinc sulfate than zinc from a chelated source to promote comparable growth when fed diets containing phytic acid. The chelate tested proved to be a safe, effective and available source of zinc for the Pacific white shrimp.

Keywords: Pacific white shrimp, Litopenaeus vannamei, chelated zinc source, phytic acid, bioavailability, cost effective feeds, growth

1. Introduction

Zinc is an essential trace element for both terrestrial and aquatic animals and plays vital roles in metabolism, structural integrity and regulatory function (Cousins, 1996; Davis and Gatlin, 1996; Watanabe et al., 1997; Underwood and Suttle, 1999; Lall, 2002; Shiau and Bai, 2009). It is estimated that proteins with zinc-binding domains account for approximately 10% of the total human proteome and it is likely that this is the case across animal species (Andreini et al., 2006). Zinc is an important component of numerous enzymes and transcription factors and plays crucial roles in cellular growth, immune function, reproduction, neural function, connective tissue metabolism and antioxidant function (Starcher et al., 1980; Coleman, 1992; Shankar and Prasad, 1998; Underwood and Suttle, 1999; Blanchard et al., 2001; Ho et al., 2003; Cousins et al, 2003). Zinc is an essential trace element for shrimp and other crustaceans (Davis et al., 1992; Shiau and Jiang, 2006) and is known to play important roles in immune function, lipid metabolism, gene expression, reproduction and growth (Mendez et al., 2001; Shiau and Jiang, 2006; Li et al., 2010).

The availability of zinc from feed ingredients such as fish meal that have historically been the main protein source in feeds is typically low due to the presence of tricalcium phosphate which interferes with the absorption of zinc (Satoh et al., 1987; Watanabe et al., 1988). The decline in fish meal use due to rising costs and limited availability has necessitated the increased use of plant proteins (Tacon and Metian, 2008; Naylor et al, 2009). However, the use of high levels of plant protein sources in feeds may result in the reduced availability of zinc and other trace minerals, due to the presence of phytic acid. Phytic acid binds with divalent trace minerals and reduces their availability to animals resulting in loss to the environment as waste (Cheryan, 1980; Davis et al., 1993; Davis and Gatlin, 1996; Li and Robinson, 1997; Helland et al., 2006). The lower availability of zinc from feeds could impact the growth and health of both cultured and wild populations and is an area of serious concern (Ahsanullah et al., 1981; Munsiri et al., 1996; Wu and Chen, 2005; Cheung and Wong, 2006; Wu et al., 2008; Azevedo et al., 2009; Wu and Yang, 2011; Umamaheshwari et al., 2011). The alter-

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native to meet the nutritional requirement for zinc in shrimp diets would be to significantly increase dietary zinc levels which would have the potential negative effect of increasing discharge of zinc into the environment.

Organic trace minerals are considered to be more available than inorganic trace minerals. There have been several studies in fish demonstrating higher availability of minerals from organic sources (Hardy and Shearer, 1985; Paripatananont and Lovell, 1995a; 1997; Apines et al., 2003; Apines-Amar et al., 2004b; Buentello et al., 2009; Shao et al., 2010). Typically organic trace minerals are more stable in the gastrointestinal tract and less susceptible to interactions and binding with antagonists as they are bound to organic ligands. Some commonly available organic trace minerals are in the form of metal proteinates, metal amino acid complexes and metal amino acid chelates. Studies have demonstrated improved bioavailability, growth and disease resistance in fish fed metal proteinates (zinc proteinate) and metal amino acid complexes (zinc methionine) compared to fish fed inorganic sources (Hardy and Shearer, 1985; Paripatananont and Lovell, 1995a;b; Buentello et al., 2009). The glycine chelates of trace minerals have also been shown to improve performance, tissue mineral retention, immune function and disease resistance in the rainbow trout despite the presence of dietary antagonist such as phytic acid and tricalcium phosphate (Satoh et al., 2001; Apines et al., 2003; Apines-Amar et al., 2004a;b) and in red sea bream (Sarker et al., 2005). More recently, divalent trace minerals chelated to HMTBa (2-hydroxy-4-methylthiobutanoic acid; hydroxy analog of methionine; Mintrex™; Novus International Inc, St. Charles, USA) have become available for use in animal feeds. The stability of these molecules makes them less available to binding to phytic acid enabling them to reach receptors in the gut wall where they are absorbed into circulation (Yi et al., 2007; Richards et al., 2010).

In a recent study Pacific white shrimp *L. vannamei* fed a diet containing a zinc-methionine complex performed significantly better than shrimp fed either a zinc-deficient control diet or diets containing zinc sulfate or zinc complexed to lysine or glycine (Lin et al., 2013). These diets used in the study were purified diets based on casein and gelatin and the response of shrimp to such sources in practical diets that contain various mineral antagonists is not fully understood. There is limited information on the availability of organic or chelated trace minerals in shrimp fed either phytic acid-containing or phytic acid-free diets. The effect of phytic acid on mineral availability has however been well documented in fish (Spinelli et al., 1983; Richardson et al., 1985; Helland et al., 2006; Laining et al., 2010) but has been less studied in shrimp. Phytic acid did not adversely affect growth in *Penaeus japonicus* but depressed growth in *L. vannamei* (Civera and Guillaume, 1989). A dietary excess of zinc was required to overcome the effects of phytic acid and to restore levels of growth observed in *L. vannamei* fed diets without phytic acid (Davis et al., 1993). Increasing dietary zinc levels in response to decreased bioavailability results in higher losses of zinc into the environment from aquatic production systems.

Therefore the objective of this study was to evaluate the effects of inorganic and chelated dietary zinc sources on performance and tissue zinc retention in Pacific white shrimp *L. vannamei* fed diets containing phytic acid.

2. Materials and Methods

2.1. Experimental diets

A base diet composed mainly of wheat starch, casein, gelatin, squid meal and soy protein isolate was formulated to be deficient in zinc (24 ppm zinc) (Table 1). The diet, formulated to contain approximately 35% crude protein and 8% crude fat, met all nutritional requirements of the Pacific white shrimp (Table 2).

The vitamin premix used in the diet was devoid of zinc and other trace minerals; copper was added separately to the diet in the form of Mintrex™ copper, manganese as manganese sulfate, potassium as potassium chloride, calcium as calcium carbonate and magnesium as magnesium oxide respectively to meet requirements for this species. All diets were mixed and pelleted at the Texas A&M AgriLife Research Mariculture Laboratory, Port Aransas, Texas. Pellets were manufactured using a laboratory scale pellet mill and pellets were dried in a forced-fan oven at 65-75°C and cooled at ambient temperature. Diets were manufactured by mixing all dry ingredients in a V-mixer followed by addition of addition of menhaden oil and deionized water to the mixture. The base diet and diets containing high levels of either zinc sulfate ($ZnSO_4.7H_2O$ reagent grade) or Mintrex™ zinc (24 ppm in the base diet and 363 ppm and 104 ppm in the zinc sulfate and Mintrex™ zinc diets respectively) were mixed at various ratios to produce diets containing intermediate levels of either source of zinc.

The basal diet and diets containing the high level of either source of zinc were analyzed for their zinc and phytic acid concentrations. Diets were then pelleted through a 3 mm die and then dried in a forced air oven overnight until dry and then crumbled and sieved to the required size. All diets were stored in a -10°C freezer until required. Two series of diets were formulated, one containing zinc from zinc sulfate ranging from 55-363 ppm (55, 80, 116, 168, 243, and 363 ppm respectively) and the other containing zinc from Mintrex™ zinc ranging from 39-104 ppm (39, 52, 65, 78 and 104 ppm respectively) (Table 3). All experimental diets also contained approximately 1.38% phytic acid which is similar to concentrations observed in commercial aquaculture diets.

2.2. Feeding trial

Thirteen diets, including the base diet and a reference diet were fed to shrimp during a growth trial that was conducted at the Mariculture Laboratory at Texas A&M University System, Port Aransas, Texas, USA. Juvenile shrimp (mean initial weight 0.40

Table 1. Composition of base diet used to evaluate efficacy of dietary zinc sources in shrimp. Values are in % on an "as fed" basis.

Ingredient	% of diet
Casein	13.30
Gelatin	13.00
Wheat Starch	29.67
Soy Protein isolate	7.00
Squid Muscle Meal	6.00
Dicalcium Phosphate	6.00
Dry Lecithin, 95%	4.00
Cellulose	3.20
Menhaden Fish Oil	2.60
Diatomaceous Earth	2.50
Potassium Chloride	2.50
Calcium Carbonate	2.20
Alginate (Manucol F)	1.60
Magnesium Oxide	1.60
Chromic Oxide	1.00
Sodium Hexametaphosphate	1.00
Phytic Acid	1.00
Soybean Oil	0.60
Vitamin Premix	0.46
Cholesterol	0.20
L-methionine	0.10
Vitamin C	0.04
Mintrex™ copper (methionine hydroxy analog chelated)	0.02
Manganese Sulfate Monohydrate	0.01
Sodium Selenite	0.00005

g) were obtained from a hatchery in Texas and assigned randomly to 26 L tanks (8 replicates per treatment) with a 20 cm water depth. Each tank was stocked with 6 shrimp that were fed 15 times daily with an automatic feeder to excess to attain a final FCR of approximately 2.0. Molts, shrimp waste and uneaten feed were removed daily. Shrimp were fed the zinc-deficient basal diet for 7 days prior to the trial to reduce tissue stores of zinc. Prior to this depletion period shrimp were fed a commercial diet while they were being acclimated to the experimental system. The study was carried out in two re-circulating systems that were supplied with filtered seawater of ambient salinity that was pumped in from the adjoining bay resulting in clean water experimental conditions with zero contribution from natural productivity.

The water temperature during the study was approximately 30°C. Water temperature, dissolved oxygen, salinity, pH and ammonia concentrations were measured on a regular basis. The experimental systems were monitored on a daily basis for dissolved oxygen (DO), salinity and temperature using a YSI 85 Meter (Yellow Springs, Ohio, USA). Ammonia (TAN), nitrite, nitrate and pH were measured on a weekly basis using standard procedures.

The growth trial was conducted for 6 weeks, and at the end of the trial shrimp were weighed to calculate weight gain. Immediately afterwards 3 shrimp from each tank were pooled together with shrimp from other tanks from within each treatment and split into 3 groups per treatment. Five shrimp from each treatment group were dissected and their hepatopancreases pooled

Table 2. Analyzed nutrient values for base diet. All values ("as fed") are as % of diet except for trace minerals which are in ppm.

Nutrient	%
Crude Protein	35.21
Crude Fat	8.31
Crude Fiber	3.97
Ash	17.12
Phytic Acid	1.38
Trace Minerals	
Copper	54
Iron	148
Manganese	40
Zinc	24

together for zinc analysis. Three shrimp from each treatment group were pooled together for whole body zinc analysis. These tissues were dried, ashed, digested with concentrated nitric acid and analyzed for zinc concentrations using ICP-AES analysis (Perkin Elmer Optima 2100DV equipped with a GEMCONE Cyclonic Spray Nebulizer; Perkin Elmer, Waltham, MA, USA). Experimental diets were similarly treated and their trace mineral concentrations determined. Proximate analysis of diets was carried out using standard methods (AOAC, 2006). Phytic acid in experimental diets was measured using ion exchange chromatography.

2.3. Data Analysis

Statistical analysis of data was carried out using SAS version 9.2 (Cary, North Carolina). One-way analysis of variance (ANOVA) was used to evaluate the effect of zinc source on response variables. Data were also analyzed using ANOVA with system and row as blocking factors. Where significant differences were detected ($p < 0.05$) a least square means (LSMeans) test was applied to separate differences between treatments. Percentage data were arc sine transformed prior to statistical analysis. Each tank was considered as an experimental unit.

3. Results

3.1. Water quality

Water temperature during the trial was maintained at 29.5 ± 0.3°C, salinity at 35 parts per thousand and DO at 5.8 ± 0.3 mg/L. Total ammonia nitrogen was 0.16 ± 0.05 mg/L, nitrite nitrogen at 0.09 ± 0.07 mg/L and nitrate nitrogen at 1.16 ± 0.59. Water pH was measured at 8.03 ± 0.08. Water quality conditions during the trial were maintained at levels optimal for this species.

3.2. Performance

The initial weight was 0.40 ± 0.003 g with no significant differences observed between treatment groups. Percent survival for all treatments was high (94-98%) with no significant differences in survival between the treatment groups (Table 4). Shrimp attained final weights of 9.55 -10.59 g and shrimp fed diets supplemented with high levels of zinc sulfate (Z243 and Z363) exhibited significantly higher final weights than the group fed the base diet. In contrast, shrimp fed much smaller levels of supplemental dietary zinc (M65 and M78) from the chelated source showed significantly higher final weights than shrimp fed the basal diet (Table 4).

Growth rates ranged from 1.60 g/wk to 1.78 g/wk (Table 4). Shrimp from treatment groups Z243 and Z363 that were fed diets supplemented with zinc sulfate exhibited significantly higher growth rates than shrimp fed the base diet. Shrimp fed considerably lower levels of chelated zinc (M65 and M78) also showed significantly higher growth rates than shrimp fed the base diet.

Biomass of shrimp per m^2 ranged from $546.8 g/m^2 - 621.6 g/m^2$ (Table 4). Shrimp from the treatment group Z243 fed a diet supplemented with zinc sulfate showed significantly higher biomass than the control group fed the base diet. Similarly, shrimp fed diets supplemented with chelated zinc (M65 and M78), but at lower levels than that in groups fed zinc sulfate supplemented diets, also exhibited significantly higher biomass than the group fed the base diet.

Final weights and other performance parameters (Table 4) indicate that 3-4 times as much zinc from zinc sulfate was required compared to zinc from the chelated source to promote a performance response of similar magnitude.

3.3. Whole body and hepatopancreas zinc concentrations

Increasing dietary zinc concentrations did not exert a consistent effect on tissue zinc concentrations (Table 5).

4. Discussion

Results from the study indicate that chelated zinc was more available than inorganic zinc to shrimp in the presence of phytic acid, a known trace mineral antagonist. Data suggest that shrimp required 3-4 times more inorganic zinc than chelated zinc to promote a comparable final weight, growth rate and biomass (Table 4). Performance in shrimp fed 243 and 363 mg/kg inorganic zinc were similar to shrimp fed 65 and 78 mg/kg of zinc from the chelated source, suggesting a higher requirement for zinc from an inorganic source in practical diets. This study

Table 3. Experimental *treatments* used to evaluate the efficacy of different sources of zinc in the Pacific white shrimp *Litopenaeus vannamei*

Zinc Source	Diet	Supplemental Zinc, mg/kg	Total Dietary Zinc Concentration, mg/kg	Analyzed Dietary Zinc Concentration, mg/kg*	Phytic Acid, %*
	Base	0 (24 mg/kg in Base)	24	27	1.38
Inorganic Zinc Zinc Sulfate (ZnSO4.7H2O)	Z55	31	55	60**	
	Z80	56	80	85**	
	Z116	92	116	125**	
	Z168	144	168	180**	
	Z243	219	243	261**	
	Z363	339	363	392	1.37
Chelated Zinc Mintrex™ Zinc (chelated with methionine hydroxy analog)	M39	15	39	46**	
	M52	28	52	63**	
	M65	41	65	80**	
	M78	54	78	97**	
	M104	80	104	131	1.37

*The base diet and diets containing high levels of either zinc source were analyzed for zinc and phytic acid concentrations

**Calculated dietary zinc values for intermediate supplemental levels of either zinc source

demonstrated differences in availability of zinc between different dietary sources and these are the first results to show better performance due to organic or chelated zinc in shrimp. There are, however, numerous studies in fish showing improved availability of organic trace minerals and their subsequent effects on performance and health (Hardy and Shearer, 1985; Paripatananont and Lovell, 1995a;b; Apines et al., 2003; Apines-Amar et al., 2004a;b; Buentello et al., 2009; Laining et al., 2010; Shao et al., 2010).

Survival rates of 93% and greater were observed for the various treatments at the end of the study (Table 4). This suggests that both zinc sources were safe at the levels tested for the conditions of this experiment. In a study with the Pacific white shrimp *Litopenaeus vannamei* that were fed zinc-supplemented diets with or without phytate, survival ranged from 75-93% and 73-90% for shrimp fed diets with and without phytate, respectively. No apparent reduction in survival was noted with increase in dietary zinc supplementation up to 200 mg zinc/kg of diet (Davis et al., 1993). In black tiger shrimp *Penaeus monodon* that were fed diets supplemented with zinc (0-120 mg/kg zinc), no differences in survival were attributable to increases in dietary zinc. However, overall survival of shrimp ranged from 62-72% and these rates were generally lower than that seen in other shrimp studies (Shiau and Jiang, 2006).

Shrimp exhibited final weights of 9.55-10.59 g at the end of the 6 wk growth trial (Table 4). The final weights of shrimp fed the base diet was significantly lower than that observed in some groups fed diets supplemented with zinc sulfate (Z243 and Z363) and chelated zinc (M65 and M78). Growth rates showed a similar pattern and shrimp from some treatment groups fed diets supplemented either with zinc sulfate (Z243 and Z363) or chelated zinc (M65 and M78) displayed significantly higher growth rates than those observed in shrimp fed the base diet (Table 4). Overall, these results indicated the need for a higher degree of zinc supplementation from an inorganic source than a chelated source to promote responses of a similar magnitude. A decreasing trend in performance was apparent at higher levels of supplementation of both zinc sources. In general shrimp from groups Z363 and M104 exhibited lower performance compared to shrimp from groups Z243 and M65 and M78 respectively, reflecting the expected quadratic response and lowered growth at higher levels of zinc supplementation.

Pacific white shrimp *L. vannamei* fed a purified diet containing zinc-methionine exhibited significantly higher growth compared to shrimp fed either a zinc-deficient control diet or diets containing zinc sulfate, zinc-glycine or zinc-lysine (Lin et al., 2013). At the end of 12 wks shrimp (initial weight 0.72g) fed the zinc-methionine diet exhibited weight gain of 1394% while those in the other treatment groups ranged from 625-1092%. The diets used in this study were purified and devoid of any mineral antagonists and the mechanism by which one dietary source was more available is unclear. In an earlier study with *L.*

Table 4. Survival (%), final weight (g/shrimp), growth rate (g/wk), and biomass in g/m2 in shrimp fed the base diet (no supplemental zinc) or different levels of supplemental zinc from either zinc sulfate (Z) or chelated zinc (M). All values are means ± SEM for eight observations. The initial weight was 0.40±0.003 g with no significant differences in initial weight between treatments. Superscripts with the same letters are not significantly different.

Diet	Dietary Zinc, ppm	Survival %	Final Weight, g	Growth Rate, g/week	Biomass, g/m^2
Base	24	95.83	9.55 a	1.60 a	546.8 a
Z55	55	95.83	10.07 abc	1.69 abc	578.6 ab
Z80	80	95.83	9.73 ab	1.64 ab	560.6 ab
Z116	116	97.92	9.94 abc	1.67 abc	585.1 ab
Z168	168	97.92	10.00 abc	1.68 abc	587.0 ab
Z243	243	97.92	10.59 c	1.78 c	621.6 b
Z363	363	93.75	10.36 bc	1.74 bc	582.9 ab
M39	39	95.83	10.17 abc	1.71 abc	583.4 ab
M52	52	95.83	9.87 abc	1.66 abc	568.1 ab
M65	65	97.92	10.43 bc	1.76 bc	610.8 b
M78	78	97.92	10.36 bc	1.74 bc	607.3 b
M104	104	97.92	9.97 abc	1.67 abc	585.5 ab
SEM		0.80	0.08	0.014	6.28

vannamei, neither final weight nor weight gain responded significantly to diets supplemented with zinc either with or without phytate (Davis et al., 1993). In shrimp fed diets without phytate, maximum weight gain was observed in shrimp fed the diet supplemented with 15 mg/kg zinc. Shrimp fed diets without phytate (0, 15, 30 & 60 mg/kg supplemental zinc) showed 1671-2523% weight gain while those fed diets with phytate (0, 60 & 200 mg/kg supplemental zinc) showed 1952-2272% weight gain. The presence of phytate did not depress growth significantly but depressed hepatopancreas zinc levels and required 200 mg/kg supplemental zinc to restore levels to those seen in shrimp fed diets without phytate (Davis et al., 1993). Similarly, in an earlier trial with the same species, deletion of zinc from the mineral premix from diets fed to shrimp did not affect growth significantly but depressed tissue zinc concentrations (Davis et al. 1992). In *P. monodon* fed diets supplemented with zinc ($0-120$ mg/kg), shrimp fed diets supplemented with $\leqslant$ 35 mg/kg zinc displayed significantly greater weight gains than shrimp fed diets supplemented with $\leqslant 17.5$ mg/kg of zinc (Shiau and Jiang, 2006). The relatively large initial weight of 0.44g and high mortality in that study may have contributed to the lower weight gains observed which ranged from 176-244%.

Tissue zinc concentrations were not responsive to changes in dietary zinc and no increase in either whole body or hepatopancreas zinc was observed with dietary zinc supplementation (Table 5). In an earlier study with *L. vannamei*, hepatopancreas zinc content was significantly affected by dietary zinc and phytate. Hepatopancreas zinc concentrations were low in shrimp fed the unsupplemened basal diet and reached a plateau at 15 mg/kg. In shrimp fed diets containing phytic acid, supplementation of 200 mg/kg was required to attain hepatopancreas zinc concentrations that resembled those measured in shrimp fed phytate-free diets (Davis et al., 1993). Hepatopancreas zinc concentrations ranged from approximately 100-150 mg/kg in shrimp fed diets that did not contain phytic acid and ranged from approximately $50-130$ mg/kg in shrimp fed diets containing phytic acid (Davis et al., 1993). These concentrations are similar to those measured in the present study (Table 5). In *P. monodon*, both whole body and hepatopancreas zinc concentrations were affected by dietary zinc supplementation. Hepatopancreas zinc concentrations increased from 0.58 mg/kg to 21.83 mg/kg when dietary zinc supplemental levels were increased from $0-120$ mg/kg. Whole body levels increased from $2-43$mg/kg with increasing dietary levels of zinc (Shiau and Jiang, 2006). In the Chinese mitten crab *E. sinensis hepatopancreas*, zinc concentrations increased from $31-132.7$ mg/kg when dietary zinc

Table 5. Whole body and hepatopancreas zinc concentrations in shrimp fed diets containing either zinc sulfate (Z) or chelated zinc (M) or an unsupplemented base diet. All values are means.

Diet	Dietary Zinc Level, ppm	Whole Body Zinc*, ppm	Hepatopancreas Zinc**, ppm
Base	24	64.05	97.55
Z55	55	69.98	129.00
Z80	80	71.15	99.80
Z116	116	71.08	99.15
Z168	168	67.18	155.00
Z243	243	68.93	90.70
Z363	363	67.58	157.50
M39	39	69.98	117.00
M52	52	66.57	116.70
M65	65	67.02	117.50
M78	78	64.85	98.50
M104	104	70.48	117.60
SEM		0.97	7.09

* whole body samples were taken from each tank and pooled into 3 samples per treatment

** hepatopancreas samples were pooled into 2 samples per treatment

levels were raised from $5 - 85$ mg/kg (Li et al., 2010). In general, tissue zinc levels from different crustacean species were in general agreement (Depledge, 1989; Davis et al., 1992; 1993; Shiau and Jiang, 2006; Li et al., 2010). In the present study, tissue zinc concentrations were not responsive to changes in dietary zinc and it is probable that tissue zinc concentrations were maintained within a certain range. With the much higher dietary supplementation of zinc from zinc sulfate as compared to the chelated source required for the greatest performance with no changes in tissue zinc, a much greater amount of zinc would be discharged into the environment using zinc sulfate versus the chelated source in shrimp feed.

Growth results from the present study suggest that chelated zinc is more available presumably because inorganic zinc is more prone to binding by phytic acid. This results in a higher requirement for inorganic zinc to produce a comparable level of growth. Phytic acid has a strong affinity for zinc and other divalent minerals which results in their being unavailable to aquatic and terrestrial animals (Cheryan, 1980; Davis et al., 1993; Davis and Gatlin, 1996; Richards et al., 2010). Phytic acid has been observed to depress growth and also lower the availability of macro minerals and trace minerals in fish and shrimp (Spinelli et al., 1983; Richardson et al., 1985; Civera and Guillaume, 1989; Paripatananont and Lovell, 1995a; Usmani and Jaffri, 2002; Portz and Liebert, 2003; Helland et al., 2006; Laining et al., 2010). In the Pacific white shrimp *L. vannamei*, addition of phytic acid to diets did not significantly depress growth but it affected hepatopancreas zinc concentrations (Davis et al., 1993). Supplementation of excess zinc to the diet overcame the presence of phytic acid in the diet and restored hepatopancreas zinc levels to those seen in shrimp fed diets without phytic acid (Davis et al., 1993). In a trial that examined the suitability of sodium phytate and disodium phosphate as dietary phosphorus sources, no differences in growth were observed when phytate was fed to the kuruma prawn *Penaeus japonicus*, however the presence of phytate depressed growth in *L. vannamei*. Diets in the present study contained approximately 1.4% phytic acid (Table 2) and this is in agreement with concentrations measured in fish and shrimp feeds from different parts of the world (Novus International Inc; unpublished results).

Results from this study suggest that chelated minerals have a lower affinity for binding with phytic acid and consequently are more available to shrimp than inorganic mineral sources. There is no well established mechanism to explain this phenomenon but we hypothesize that zinc and other divalent trace elements form a very stable structure with the ligand 2-hydroxy-4-methylthio-butanoic acid (HMTBa) that makes the minerals less susceptible to binding with and interference from antagonistic factors. These molecules are therefore able to reach transporters in the epithelial layer of the gut where they are trans-

ported into the enterocytes and circulation. The chelated zinc molecule is then cleaved at the gut wall with the mineral ions being transported and the HMTBa diffusing passively into the epithelial layer. The HMTBa is converted into L-methionine via a series of enzymatic steps and is utilizable by the animal (Dibner and Knight, 1984; Dibner et al, 1987; Forster and Dominy, 2006; Yi et al., 2007; Richards et al., 2010). This enables the chelated zinc source to be more available despite the presence of antagonistic factors and as a result less supplemental zinc is required to satisfy the requirement of the animal and promote growth. Higher zinc availability also has implications for the health of shrimp and that of the environment. There is concern over discharge of trace minerals into the environment from aquaculture farms and higher zinc bioavailability can ensure lower emissions into the environment. Furthermore waterborne zinc can exert an influence on the health of both cultured and wild populations of aquatic animals.

In conclusion, performance results suggest that a chelated zinc source was more available than zinc from an inorganic source to shrimp fed diets containing phytic acid. Results indicate that 3-4 times the quantity of inorganic zinc was required to promote comparable levels of growth to that observed in shrimp fed a chelated source of zinc. The study showed that zinc chelated with HMTBa was a safe, effective and highly available source of dietary zinc for the Pacific white shrimp *L. vannamei*.

5. Acknowledgments

The study was supported by Novus International, Inc. and by Texas A&M AgriLife Research, Texas A&M University System.

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