Asian Fisheries Science **13**(2000): 201-207
Asian Fisheries Society, Manila, Philippines

Copper, Manganese and Zinc Requirements in the Diet of Shrimp *Penaeus indicus*

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Abstract

Dietary requirements of copper, manganese, and zinc were investigated for the Indian white shrimp *Penaeus indicus* using purified diets. Feeding trials conducted on shrimp with an average weight of 70 mg for 45 days have shown that without supplemented copper, the diet produced higher growth ($p > 0.05$). However, the diet with 22.7 mg% copper gave the lowest feed conversion ratio (FCR) of 2.14 ($P > 0.05$) and higher survival rate (81.3%). Copper content in whole body of shrimp increased with dietary copper. Shrimp fed with diets having different levels of manganese grew better on the diet (control) that had 0.21 mg of manganese per 100 g. Higher levels of dietary manganese suppressed growth ($P > 0.05$). However, food conversion ratio (FCR) and survival rate improved when the diet contained 1.9 to 2.6 mg% manganese. In the case of zinc, the growth of shrimp improved with dietary zinc up to 23.6 mg% and declined thereafter ($p > 0.05$). However, the diet with 38.6 mg% zinc produced low FCR ($p > 0.05$). Zinc concentration in shrimp body increased with the dietary levels of the element, though the body ash showed a decreasing trend.

Introduction

There are many inorganic elements in the body of shrimp associated with the skeletal structure and biochemicals involved in vital physiological functions. Some of them are required in major quantities such as calcium, phosphorus, potassium, magnesium, etc. However, elements like copper, manganese, zinc, etc. are needed only in traces and are referred to as micronutrients. Although shrimps are capable of extracting some of the elements from water, they do respond to dietary sources (Deshimaru and Yone 1978; Kanazawa et al. 1984; Davis et al. 1992 and 1993). Since these micronutrients are essential, their absence in the diet may lead to deficiency disease. It is therefore necessary to understand their dietary requirement in formulating balanced feeds. In the present study, the effects of different levels of copper, manganese, and zinc in diet on the growth, FCR, and survival of Indian white shrimp *Penaeus indicus* were investigated.

Materials and Methods

Formulation and preparation of test diets

Semipurified diets were formulated using a basal diet mixture consisting of fibrin (bovine blood-SIGMA), albumin (egg-BDH), cod liver oil, maltose-sucrose-starch (1:1:1 ratio), and other additives (Table 1). Each mineral element was tested at seven dietary levels. Copper sulphate, manganese sulphate, and zinc chloride were used as sources of the respective elements. The diets in each group were evaluated separately in three different experiments and the dietary requirement shown for the mineral by the shrimp was included in the subsequent groups of test diets. The composition of the diets is shown in table 2.

The diets were prepared as dry pellets of 3 mm diameter after grinding solid ingredients to pass through a 300 micron sieve, they were mixed and homogenized with binder solution (made in 30 ml hot water for 100 g diet), steamed for 5 minutes and extruded through a hand pelletizer. The pellets were dried at 70°C and crushed to granules of approximately 1 mm size, which were used in the feeding trials.

Feeding experiments

Feeding experiments with test diets were conducted on hatchery reared (from single brood) young ones of shrimp *Penaeus indicus* with an average live-weight of 70 mg. The shrimps were stocked in circular tanks containing 10 l of saline water. In each tank eight shrimp were stocked and there were three replicates for each treatment. Initially the shrimp were fed at the rate of 20% of their body weight divided in two doses daily. The quantity of diet was later regulated based on the left-over diet every day. The tanks were cleaned daily by removing the sediments and water was totally replaced with a fresh batch of water. The duration of the feeding trial was 45 days. The salinity of the water used in the experiments was 20.8 ± 1.0 ppt. The pH, dissolved oxygen, and temperature of water were 7.98 ± 0.2, 4.4 ± 0.4 mg l^{-1} and 28.5 ± 0.5°C respectively.

Table 1. Composition of basal diet mixture.

Ingredients	g·100 g diet
Fibrin (blood)	32.0
Albumin (egg)	8.0
Maltose	11.0
Sucrose	11.0
Starch	11.0
Cod liver oil	6.0
Glucosamine HCl	0.8
Cholesterol	0.5
Vitamin mix.*	2.7
Calcium carbonate	1.3
Potassium dihydrogen Orthophosphate	4.4
Binder (sodium alginate)	3.0
Total	91.7
Crude Protein (analysed%)	39.87

*Vitamin mixture (g·100 g): Ascorbic acid 2.0, choline chloride 0.12, cyanocobalamine 0.00008, folic acid 0.08, nicotinic acid 0.04, pantothenic acid (calcium salt) 0.06, para-aminobenzoic acid 0.01, pyridoxine hydrochloride 0.012, riboflavin 0.008, thiamin hydrochloride 0.004, biotin 0.00004, ß-carotene 0.0096, calciferol 0.0012, inositol 0.2, menadione 0.004 and α-tocopherol 0.029.

Analysis of samples

The mineral elements in the diets and animal tissues were analyzed using the Atomic Absorption Spectrophotometer (AAS) of Perkin-Elmer, Model 2380 in air acetylene flame. Samples of shrimp whole body were prepared by taking three representative samples for each treatment, washed with deionized water and oven dried. Diets and animal samples for trace element analysis were prepared following the dry ashing method as per the procedure of the Association of Official Analytical Chemists (AOAC) 1984. The trace elements were analyzed in AAS following the methods of AOAC (1984). Crude protein and ash were estimated also according to standard AOAC (1984) methods.

Statistical analysis

Data obtained on growth and FCR in feeding experiments were subjected to ANOVA following Snedecor and Cochran (1973) and the means of different treatments were compared following the Least Significant Difference (LSD) method.

Table 2. Composition of test diets.

Copper diets	C1	C2	C3	C4	C5	C6	C7
Basal diet mix (g)	91.7	91.7	91.7	91.7	91.7	91.7	91.7
Copper sulphate (g)	0.0	0.0070	0.0105	0.0140	0.0420	0.0700	0.1400
Cellulose (g)	8.3	8.293	8.289	8.286	8.258	8.230	8.160
Total copper content (Analyzed) (mg)	2.4	4.2	5.5	7.1	10.2	13.6	22.7
Manganese diets	M1	M2	M3	M4	M5	M6	M7
Basal diet mix (g)	91.7	91.7	91.7	91.7	91.7	91.7	91.7
Manganese sulphate (g)	0.0	0.002	0.003	0.004	0.005	0.006	0.008
Copper sulphate (g)	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Cellulose (g)	8.230	8.228	8.227	8.226	8.225	8.224	8.222
Total manganese content (Analyzed) (mg)	0.21	0.69	1.00	1.30	1.60	1.90	2.60
Zinc diets	Z1	Z2	Z3	Z4	Z5	Z6	Z7
Basal diet mix (g)	91.7	91.7	91.7	91.7	91.7	91.7	91.7
Zinc chloride (g)	0.0	0.02	0.04	0.06	0.08	0.10	0.12
Copper sulphate (g)	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Cellulose (g)	8.23	8.21	8.19	8.17	8.15	8.13	8.11
Total zinc content (Analyzed) (mg)	6.0	14.1	18.9	23.6	30.4	38.6	50.2

Results

Experiment 1

Shrimps fed with diet C1 apparently showed the highest increase in weight but growth decreased with an increase in dietary copper level (Table 3), though the differences among the treatments were not statistically significant (P > 0.05). However, the FCR showed a slight improvement when the diet had 13.6 mg% copper and showed further improvement when the copper concentration in the diet was 22.7 mg% (P > 0.05). Survival of shrimp was also highest on this diet. The total ash content in the body of shrimp fed with diets having various levels of copper at first decreased from 16.7% to 14.0% and increased again to 17.7%. The copper concentration in the body of shrimp however, showed increasing trend with the dietary copper level (Table 3). The results indicated that dietary copper levels of 13.6 to 22.7 mg% could help only in improving the FCR and survival of shrimp.

Experiment 2

In the case of manganese diets, the shrimp fed with diet M1 with no manganese added, showed better growth (p > 0.05) (Table 3). This diet had 0.21 mg% of manganese originally. The growth of shrimp was suppressed

Table 3. Results of feeding trials with test diets to juvenile *Penaeus indicus* for 45 days.

Copper Diets	C1	C2	C3	C4	C5	C6	C7
Weight increase %	601.4	497.8	549.3	408.2	420.7	435.0	487.1
FCR	2.42	2.48	2.85	2.53	2.82	2.27	2.14
Survival %	68.9	75.0	75.0	75.0	68.0	66.7	81.3
Total body ash %	16.7	15.5	14.9	14.0	16.8	17.7	17.0
Total body copper (Analyzed) mg %	36.0	36.1	37.2	39.6	38.9	38.8	43.0
Manganese Diets	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**
Weight increase %	478.1	340.0	340.0	312.9	341.4	370.7	347.9
FCR	2.40	3.02	4.00	4.79	3.51	3.08	3.75
Survival %	50.0	43.8	48.5	54.2	58.3	62.5	79.2
Total body ash %	17.5	17.2	17.3	18.0	19.8	21.0	19.4
Total body manganese (Analyzed) mg%	0.55	0.90	1.11	1.33	1.35	1.40	1.75
Zinc Diets	**Z1**	**Z2**	**Z3**	**Z4**	**Z5**	**Z6**	**Z7**
Weight increase%	340.0	418.4	429.3	441.4	432.1	419.3	284.3
FCR	2.70	2.66	2.76	2.81	2.94	2.30	3.03
Survival %	79.2	79.2	70.8	70.8	66.3	56.3	81.3
Total body ash %	15.0	16.8	14.9	12.5	14.5	13.0	14.3
Total body zinc (Analyzed) mg %	23.3	26.3	28.5	32.0	35.5	33.3	37.8

ANOVA Weight increase and FCR not significant at 5% (P > 0.05) and FCR increased (P > 0.05) with dietary manganese levels up to 1.6 mg%. The survival of shrimp improved as dietary manganese levels increased from 1.3 mg% to 2.6 mg%. Total body ash of shrimp fed with different diets (Table 3) and body manganese concentrations increased with an increase in dietary manganese.

Experiment 3

The shrimp fed with diet Z4, having 23.6 mg% zinc showed the highest growth (P>0.05) compared to that of the other diets (Table 3). However, diet Z6 having 38.6 mg% of zinc recorded the lowest FCR of 2.3 (P>0.05) among the diets tested. Although the survival of shrimp fed with diet having 50 mg% zinc was high, the growth was poor. The total body ash content of shrimp slightly declined (Table 3), but the body zinc levels gradually increased with dietary zinc (Table 3). Taking all the aspects of performance of the diets, it may be inferred that the zinc requirement in the diet of *P. indicus* lies between 23.6 mg% and 38.6 mg%.

Discussion

In crustaceans, it is well known that copper is associated with haemocyanin (hemolymph), which participates in oxygen transport. The copper content found in the haemocyanin of decapods crustaceans such as *Homarus* and *Limulus* is about 0.25%. This is a remarkably higher concentration (Goodwin 1960) compared to the copper content generally found in seawater (0.01 microgram per 100 ml). Whether copper is required in the diet of shrimp or they are capable of absorbing this element from the surrounding water to meet the requirement has been a subject of debate. There are evidences that crustaceans do absorb copper from water (Bryan 1968; White and Rainbow 1982; Subhash Chander 1986). Deshimaru and Yone (1978) opined that copper may not be required in the diet of shrimp. However, some of the natural foods such as *Artemia* nauplii, rotifers (*Brachionus* sp), *Daphnia*, and *Tigriopus* used for feeding shrimp larvae and postlarvae contain copper ranging from 11-159 mcg, 4-23 mcg, 11-13 mcg and 18-22 mcg respectively (Watanabe et al. 1978), which shows that diet is an important source of copper for shrimp. Kanazawa et al. (1984) recommended 0.6% copper in the diet of *Penaeus japonicus*. Davis et al. (1992) indicated a dietary need of copper for *Penaeus vannamei* and subsequently recommended a dietary requirement of 32 mg copper per kg diet (Davis et al. 1993). In the present study, addition of copper to diet did not improve the growth of *P. indicus* but the FCR and survival rate were better when the diet contained 13.6 to 22.7 mg% copper. This is much lower than the dietary copper recommended for *P. japonicus* (Kanazawa et al. 1984) and higher than the dietary requirement shown by *P. vannamei* (Davis et al. 1993). It is suggested that a dietary supplementation of 13.6 to 22.7 mg copper per 100 g diet is beneficial for the shrimp *P. indicus*.

The body concentration of manganese in *P. indicus* increased progressively with the dietary levels of the element. In line with these results, Davis et al. (1992) observed decreased carapace concentration of manganese when the element was deleted from the diet of *P. vannamei*. The growth of *P. indicus* fed on diets supplemented with manganese did not improve in the present study. However, the survival of shrimp showed improvement with dietary manganese. These results indicate that there may be a dietary requirement for manganese for this shrimp, which may be very low. The control diet, that gave better growth and FCR among the diets tested, had 0.21 mg% manganese that may be adequate for this purpose. Kanazawa et al. (1984) also found no improvement in the growth of *P. japonicus* fed on diets supplemented with 0.001 and 0.01% of manganese. The results of the present study are in agreement with these findings. However for better survival, dietary levels of 1.9 to 2.6 mg% manganese are suggested for *P. indicus*

The results with zinc supplemented diets indicated that zinc may be required in the diet of *P. indicus* at 23.6 to 38.6 mg% which is within the range of zinc requirement of 34.5 mg% recommended for *P. japonicus* by Deshimaru and Yone (1978). Davis et al. (1993) found that the zinc requirement in the diet of *P. vannamei* is 15 mg kg^{-1} diet in the absence of dietary phytate. But in the presence of phytate (1.5%), the requirement of zinc was found to be as high as 200 mg kg^{-1} diet to obtain the same growth increment. This is slightly lower than the range of dietary requirement of zinc shown by *P. indicus*. In their preliminary studies, Davis et al. (1992) observed that deletion of zinc from the diet did not affect growth but depressed tissue mineralization of zinc. In the present study, the body concentration of zinc also increased with dietary zinc levels. The conventional live food organisms used for feeding young shrimp are rich in zinc (160 to 960 mcg g^{-1} in *Artemia*; 43 to 99 mcg g^{-1} in rotifers; 81 to 406 mcg g^{-1} in *Tigriopus*) (Watanabe et al. 1978). This suggests that food is a source of zinc for shrimp and further strengthens the present findings.

Acknowledgments

The author is grateful to Dr. P. Vedavyasa Rao, former Director, Central Marine Fisheries Research Institute, Cochin for his valuable guidance in this work. Thanks are also due to Dr. E.G. Silas, former Director, CMFRI, Cochin for his encouragement.

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Manuscript received 25 June 1998; Accepted 22 March 2000.