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Effect of Pyridoxine on Growth, Metabolism and Cellular Activity of *Macrobrachium rosenbergii*

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Effect of pyridoxine on growth, metabolism and cellular activity of freshwater prawn *Macrobrachium rosenbergii* was studied. Post-larvae (PL-10) of *M. rosenbergii* were fed with clam meat containing various concentrations of pyridoxine. After 30 days RNA and DNA of the abdominal tissues were estimated. Length, weight and RNA to DNA ratio increased significantly with increasing concentrations of pyridoxine. The effect of pyridoxine on the metabolic enzyme, malate dehydrogenase, was also studied. V_{max} showed a significant decrease and the (K_m) showed a significant increase in experimental groups compared to control.

Key words: ***M. rosenbergii*, pyridoxine, feeding, growth**

Knowledge on basic nutritional requirements and field applications on specific diet for growth and development is restricted to a few species of crustacea (Kanasawa, 1984). Several diets with *Spirulina platensis* as protein source have been evaluated in *M. rosenbergii* (Sherief, 1989). However, information on vitamin requirement and its importance in feeds is scarce.

Pyridoxine in its coenzymatic form pyridoxal-5-phosphate (PLP) is involved in several reactions mainly related to amino acid metabolism. PLP is a cofactor in aminotransferase reactions. PLP dependent reactions studied in the nervous system are involved in the catabolism of various amino acids. The putative neurotransmitters dopamine, norepinephrine, serotonin, histamine, γ-aminobutyric acid and taurine as well as the sphingolipids and polyamine biosynthesis are catalysed by PLP dependent enzymes (Dakshinamurti *et. al.*, 1985). Malate dehydrogenase (MDH) is one of the important tricarboxylic acid cycle enzymes, which determine the concentration of oxaloacetate, one of the starting substrate. The enzyme activity is an index of metabolic state of an organism. The results of experiments conducted to study the role of pyridoxine (Vitamin B_6) on metabolism, cellular activity and growth of *M. rosenbergii* were reported in this paper.

Materials and Methods

Post-larvae (PL-10) of *M. rosenbergii*, from the ponds of College of Fisheries, Panangad, were acclimatised to the laboratory conditions. The individuals weighing 30 mg and 1.2 cm of length were used for the experiments. Experimental animals were maintained under 12 h light and 12h dark cycles. Pyridoxine hydrochloride and

DNA were of Sigma Chemical Co. USA and all other biochemical used were of analytical grade. Clam meat purchased from local markets was sun dried and pulverised. Pyridoxine at the levels of .18 mg (1X), 36 mg (2X) and 180 mg (10X) incorporated into 100g of clam meat powder and used for detailed study after screening various concentrations of pyridoxine. Pulverised clam meat without pyridoxine was used as control diet. 10% starch was used as binding agent.

One set of experiment was conducted in glass tanks of capacity 50 litres having bio-filter facility and continuous aeration. The post larvae were randomly divided into four groups, each group consisting of 50 numbers and fed with 1X, 2X, 10X and control feed. Feeding was done once a day in the evening *ad libitum*. After 30 days the prawns were harvested, weight and length were measured, stored at -70°C and used for various biochemical studies.

The second set of experiment was conducted in plastic containers of 2 litre capacity with continuous aeration. The post larvae were randomly grouped into four, each group consisting of 5 numbers. They were grown in water containing no pyridoxine (A), 1 mg (B), 5 mg (C) and 10 mg pyridoxine (D) per 100 ml after initial screening of various concentrations.

DNA was extracted according to the method of Schneider (1957) with modification. Cephalothorax was removed and the abdominal tissues were used for assay. A 20% homogenate of the tissues in cold distilled water was mixed with ice cold 20% trichloroacetic acid (TCA), centrifuged to remove the acid solubles and the residue was washed with equal volume of ice cold 10% TCA. Phospholipids were removed from the residue by washing twice with two volumes of ice cold 95% ethanol. Nucleic acids were extracted from the lipid free residue by extracting twice with minimum quantity of 5% TCA heated for 10 minutes at 90°C and centrifuged. The supernatants were pooled to get the nucleic acid fraction. DNA content was quantified by diphenylamine procedure (Racker, 1952).

Acid soluble components and lipids were removed from the tissue homogenate as done for DNA extraction. The residue was suspended in equal volume of 0.5N NaOH and incubated for 18 h at 37°C. The suspension was acidified with TCA and the acid soluble fraction in the NaOH digest was used for RNA estimation by orcinol reagent (Racker, 1952).

Cytoplasmic malate dehydrogenase (S-MDH) activity was measured in the crude extract of the abdominal tissues of the prawn (Mehller *et. al.*, 1948). Kinetic parameters, V_{max} and K_m, were calculated from the data of S-MDH activity measured at substrate concentrations of 0.03 mM, 0.119 mM and 1.9 mM of oxaloacetate. Protein concentration was estimated using bovine serum albumin as the standard (Lowry *et. al.*, 1951). Statistical evaluations were done by ANOVA test using INSTAT (Ver.2.04a) computer programme.

Results and Discussion

The length and weight of prawns fed on pyridoxine incorporated diets increased significantly compared to control (Table 1). Maximum increase in length and weight was noticed in 2X (36 mg pyridoxine/100g clam meat) and the increase in length

was significant compared to 1X and 10X. Mortality rate was less than 5% in all experimental groups. RNA to DNA ratio in all experimental groups showed a significant increase compared to control. The ratio was almost same for 2X and 10X. The ratio of RNA to DNA in the group 1X was significantly lower than that of 2X (Table 1).

Table 1. Length, weight and RNA to DNA ratio of *M. rosenbergii* after 30 days feeding

Experiment	Length, cm	Weight, mg	RNA : DNA
Control	1.64±0.07	53.22±2.95	2.39±1.11
1 X	2.09±0.06**	88.05±5.06**	4.31±0.26*
2 X	3.17±0.19***@	140.12±16.66***@	6.46±0.13** $
10 X	2.11±0.05**#	91.45±11.64* £	6.45±0.17***

*P<0.05, **P<0.01 and ***P<0.001 when compared with the control; @ P<0.001 and $ P<0.05 when compared with 1 X; # P<0.001 when compared with 2 X; £ P<0.01 when compared with 2 X; Values are ± SEM of 6-8 separate determinations

The V_{max} of S-MDH significantly decreased (P< 0.001) and K_m significantly increased (P< 0.001) in all the three pyridoxine treated groups compared with the control (Table 2). The V_{max} of group C showed a significant decrease (P< 0.05) compared to group B but the K_m did not show significant variation between C and D. When group D was compared with group C, the V_{max} of the enzyme remained unchanged while the K_m showed a significant increase (P< 0.001).

Table 2. Kinetic parameters of S-MDH of *M. rosenbergii* after 10 days feeding

Experiment	V_{max} (Units/mg protein)	K_m (μM)
Group A	369.57±12.29	96.67±14.53
Group B	312.56±5.17***	231.67±10.53***
Group C	278.80±7.94***@	236.67±12.02***
Group D	295.53±8.98***	383.33±14.53***#

***P<0.001 when compared with control; @ P<0.001 when compared with Group B; # P<0.001 when compared with Group C; Values are ± SEM of 6-8 separate determinations.

Pyridoxine in its coenzymatic form, pyridoxal-5-phosphate (PLP), enhance the decarboxylation of ornithine to putrescene and it is also involved in the biosynthesis of 5-hydroxytryptamine which are known mitogenic substances (Boggust and Al-Nakib, 1986; Sudha and Paulose 1998). The 10X group showed a decrease in the rate of growth compared to 2X group. Pyridoxine at higher concentrations is reported to inhibit DNA polymerase in adenovirus (Monaghan *et. al.*, 1996). This may be the reason for the decrease in the growth rate observed in 10X group. RNA to DNA ratio is an effective means of studying cellular activity in developing organism (Clemmenson 1989; Hovenkamp, 1990) and can be used as an index of growth (Bulow, 1987). The DNA content per somatic cell is constant within a species (Leslie, 1955), while the RNA content is directly related to the rate of protein synthesis (Ikeda, 1989).

The results suggest that with increasing concentration of pyridoxine, the RNA to DNA ratio is also increasing, but the ratio was unchanged in 10X compared to 2X. This denotes that increasing pyridoxine concentration to 180mg (10X) is ineffective in changing the cellular activity. The decrease in the V_{max} and increase in the K_m of the S-MDH with increasing concentrations of pyridoxine indicate the decrease in the activity and the decreased affinity of this enzyme. The results show that pyridoxine is inhibiting S-MDH activity. This effect was confirmed by comparing the activities of the enzyme with and without PLP added to the reaction mixture. PLP and pyridoxal are reported to inhibit glutamate dehydrogenase by forming an amine with the lysine residue of the enzyme (Anderson *et. al.*, 1996). A similar mechanism of PLP inhibition of the S-MDH activity and the decrease in affinity of MDH to its substrate is suggested from the results. This decrease in activity will result in the accumulation of oxaloacetate. As the experimental animals are in the actively growing period, energy requirement for body build up is more. The accumulated oxaloacetate may be facilitating the energy production by entering the TCA cycle. Thus, the results of the present study highlight the stimulatory effect of pyridoxine on growth, metabolism and cellular activity of *M. rosenbergii* and 36 mg of pyridoxine supplemented to 100g of clam meat was found to be of significance to the growth.

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