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Vitamin C (Ascorbyl 2 Polyphosphate) Requirement of Freshwater Prawn *Macrobrachium rosenbergii* (de Man)

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Abstract

Vitamin C requirement of the giant freshwater prawn *Macrobrachuim rosenbergii* (de Man) was studied by incorporating graded levels of ascorbyl 2 polyphosphate (ApP) in semi purified diets to provide 50, 100, 150, 250 and 500 mg ascorbic acid equivalent (AAE)$\cdot$kg^{-1} diet. Prawns with 74 ± 16 mg initial body weight were used for the study. After 49 days of growth experiment, the lowest survival rate ($p < 0.05$) was recorded in 0 and 50 mg AAE$\cdot$kg^{-1} diet (36.7 and 40% respectively). Mortality was mostly found associated with the ecdysis. Highest survival rate was recorded in diets having 150 and 500 mg AAE$\cdot$kg^{-1} diet (86.67%) followed by 250 mg AAE$\cdot$kg^{-1} diet (80%). A direct relationship was observed for whole body ascorbic acid (WBAA) content of prawns and dietary levels of vitamin C in feed. At the termination of the study, statistically significant differences were observed ($p<0.05$) in the WBAA content of prawns fed with different treatments. The results of the present study show that 135 mg AAE$\cdot$kg^{-1} diet is required for normal growth and survival of juvenile *M. rosenbergii*.

Introduction

Vitamin C is essential for normal growth, immunity and reproduction in finfish and shellfish (Deshimaru and Kuroki 1976, Guary et al. 1976, Lightner et al. 1977). Classical vitamin C deficiency syndromes such as black lesions below the exoskeleton, chronic soft shelling, opaque whitish muscle, big head with flapped gill cover, incomplete moulting and increased rates of mortality have been reported in fish and shrimp (Deshimaru and Kuroki 1976, Guary et al. 1976, Lightner et al. 1977, Shigueno and Itoh 1988, Boonyaratpalin and Phongmaneerat 1995). L-ascorbic acid is extremely labile and the rate of degradation is a function of storage time, the effect of temperature, oxygen, pH and light (Herreid et al. 1952, Wanninger 1972). Attempts have been made to

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increase retention period of ascorbic acid activity in fish and prawn feeds by using alternate forms of vitamin C such as coated L-ascorbic acid (Hilton et al. 1977, Adams 1978). The third generation vitamin C products are derivatives of vitamin C essentially, in which the reactive hydroxyl group (OH) at the C2 position is protected through esterification by replacing with a sulphate or phosphate group. Since the derivatives of ascorbic acid are relatively resistant to heat and oxidation (Shigueno and Itoh 1988), it is advantageous to use these forms in prawn feed. Ascorbyl 2 poly phosphate (ApP) has already proven its ability to prevent ascorbic acid deficiency syndrome in *Litopenaeus vannamei* (Kontara et al. 1997) and *Penaeus monodon* (Chen and Chang 1994, Hari and Kurup 1998).

In shrimp aquaculture, the required quantity of ascorbic acid to be fed through artificial feeds depends mostly upon both the stocking density of the cultured species and the physiological state of the prawn. To maximize the yield in grow-out it is necessary to optimize the source and quantity of vitamin C derivatives in aquaculture prawn feed. The cost of including vitamin C in prawn feeds is very high and since the economic viability of prawn farming greatly depends upon the cost of the feed and its nutritional quality, it is essential to formulate a supplementary feed with the minimum cost. Since optimization of the quantity of vitamin C is a prerequisite for the formulation of a cost effective and efficient pelleted feed, present work attempts to determine the quantitative requirement of ascorbyl 2 polyphosphate as the vitamin C source in the feed for the giant freshwater prawn *M. rosenbergii.*

Materials and Methods

The feeding experiment conducted followed the randomized block design to eliminate the influence of position and place of the tanks among different treatments. For each set of treatment, triplicates were maintained in aquamarine colored Fiber Reinforced Plastic (FRP) tanks of 100 l capacity. *M. rosenbergii* juveniles were reared at the Hatchery Complex of the School of Industrial Fisheries for one month (initial weight = 74 ± 16 mg, initial length = 3.44 ± 0.23 cm) and were stocked at 10 prawns per tank. Water was exchanged 50% daily and a 12 h light and 12 h dark photoperiod was maintained throughout the experimental period. Prawns were fed daily with formulated experimental diets at 12% of their body weight; feeding frequency was twice a day (1100 and 2100 h). Unconsumed feed and egested fecal matter were removed at the time of water exchange. An electronic balance with precision up to 0.0001 g was used to weigh prawns; body length was measured to 0.01 mm using a dial caliper. The experimental animals were closely observed for physical abnormalities and survival was recorded through daily counts. The duration of the experiment was 49 days.

Eight semipurified diets were formulated based on the formula shown in table 1 to provide 40% crude protein and 7% crude lipid. The control diet was devoid of any vitamin C. A vitamin premix without ascorbic acid was used in all the experimental diets. Graded levels of ascorbyl 2 polyphosphate (ApP) containing 35% ascorbic acid activity was added to the semi purified diet to provide 50, 100, 150, 250 and 500 mg AAE·kg^{-1} diet respectively. The experimental feeds were stored in airtight plastic containers and kept at -4^0C.

At the termination of the feeding trials, prawns were weighed and samples were prepared by homogenizing in 5 ml ice cold 5% trichloroacetic acid (TCA) for the WBAA content analysis of the prawns. The homogenates were centrifuged for 20 min at 3000 rpm. Ascorbic acid content of the supernatant was analyzed using DNPH method described by Omaye et al. (1979) and expressed as µg·g^{-1} wet body weight of prawn. Water samples from the experimental tanks were also analyzed for dissolved oxygen and total ammonia nitrogen (APHA 1992) at weekly intervals. Temperature and pH were monitored daily using a mercury thermometer and pH meter respectively.

Growth, survival and WBAA content of prawns fed with graded levels of ascorbyl 2 polyphosphate were statistically evaluated following analysis of variance (ANOVA) and difference between means were analyzed by Duncan's multiple range test using SPSS 7.5 for Windows. Vitamin C requirements were estimated by fitting the broken line model to the survival data (Robbins et al. 1979).

Results

Weekly monitoring of water quality parameters showed that dissolved oxygen ranged from 5.1 to 7.2 mg·l^{-1}, while pH was in the range of 7.4 to 8.3 and the levels of ammonia measured were less than < 0.1 mg·l^{-1}. Water temperature was observed to be in the range of 27 to 29^0C.

Proximate compositions of the experimental feed used are presented in table 2. Moisture and protein content of the experimental diets ranged from 8.57 to 9.25% and from 39.56 to 40.91% respectively. Crude lipid content varied from 7.3 to 8.11%. Ash and crude fiber content ranged from 1.88 to 2.38%

Table 1. Composition of experimental diets (g·100 g $diet^{-1}$)

Casein (vitamin free)	35
Starch	23
Lipid mix[a]	7
Aminoacid mix[b]	3
Dextrin	5
Gelatin	5
Sodium citrate	0.3
Mineral and vitamin mix[c]	2
Choline chloride	1
Chitosan	0.8
Vitamin C	—
Cholesterol	0.5
Carboxy methyl cellulose	1
Alpha cellulose	16.4

[a]Lipid Mix-1:1 ratio of Cod liver oil and Sunflower oil
[b]Amino acid Mix-1:1 ratio of Glycine and Betaine
[c]Mineral and vitamin mix (mg.g^{-1} mineral and vitamin mix) Vitamin A, 625IU; Vitamin D3, 6.25IU; Vitamin E, 0.25 mg; niacinamide, 22.5 mg; thiamin mononitrate, 30 mg; Riboflavin, 30 mg; Folic acid, 50 mg; Biotin, 10 mg; Pyridoxine HCl, 9 mg; D Pantothenate, 37.5 mg; Cynocobalamine, 0.45 mg; Ca, 280 mg; P, 120 mg; Cu 0.2 mg; I, 1 mg; Fe, 6 mg; Mn, 1.2 mg; Se, 0.01 mg; Zn, 2 mg

and from 2.68 to 3.22% respectively. The NFE content did not vary much and ranged from 37.69 to 41.79%.

Increase in body length, weight gain, specific growth rate and survival rate of *M. rosenbergii* juveniles fed with diet containing different levels of vitamin C are presented in table 3. A significantly higher ($p < 0.05$) increase in length was recorded in diets supplemented with vitamin C levels of ≥ 150 mg

Table 2. Proximate composition of experimental diets containing different dietary levels and sources of vitamin C

	APP 0	APP 50	APP 100	APP 150	APP 250	APP 500
Moisture (%)	8.57±0.86	8.70±0.75	8.56±0.54	9.24±1.02	8.97±0.98	8.63±0.74
Crude protein (%)	40.91±1.20	40.93±1.25	40.2±0.85	39.90±1.35	39.56±0.68	40.16±1.06
Crude lipid (%)	7.78±0.45	7.50±0.86	7.98±0.45	7.85±0.84	8.11±0.82	7.37±0.64
Ash (%)	2.19±0.11	1.88±0.24	2.38±0.01	2.15±0.12	1.99±0.14	2.21±0.02
Crude fiber (%)	2.87±0.12	2.68±0.24	2.90±0.12	2.92±0.02	2.59±0.05	3.01±0.13
Nitrogen Free Extract (%)	37.69±2.12	38.32±1.54	37.96±2.45	37.94±3.01	38.78±2.45	38.61±2.65

Table 3. Weight, length, S.G.R, survival rate and whole body ascorbic acid of juveniles *M. rosenbergii* fed with graded levels of ascorbyl 2 poly phosphate (Mean±S.D) for 49 days.

	APP 0	APP 50	APP 100	APP 150	APP 250	APP 500
Initial length (cm)	2.43±0.04	2.37±0.53	2.36±0.10	2.41±0.09	2.34±0.07	2.52±0.14
Final length (cm)	2.89±0.12	32.89±0.03	3.00±0.04	3.33±0.02	3.19±0.12	3.29±0.11
Increase in length (cm)	$0.47±0.08^{a}$	$0.52±0.04^{a}$	$0.64±0.14^{ab}$	$0.92±0.11^{c}$	$0.85±0.14^{c}$	$0.78±0.04^{bc}$
Increase in length (%)	$19.24±3.10^{a}$	$22.04±2.16^{a}$	$27.49±7.51^{a}$	$38.51±5.88^{c}$	$36.20±6.55^{bc}$	$30.92 ±3.22^{bc}$
Initial weight (g)	0.07±0.01	0.07±0.01	0.07±0.02	0.08±0.02	0.06±0.01	0.08±0.02
Final weight (g)	0.14±0.02	0.16±0.01	0.17±0.01	0.23±0.02	0.21±0.02	0.22±0.02
Weight gain (g)	$0.07±0.01^{a}$	$0.09±0.01^{a}$	$0.10±0.02^{a}$	$0.15±0.04^{b}$	$0.14±0.01^{b}$	$0.15±0.02^{b}$
Increase in weight (%)	$112.62±7.65^{a}$	$127.79±38.17^{a}$	$142.34±54.04^{a}$	$214.57±33.14^{a}$	$224.39±17.48^{a}$	$211.12 ±35.16^{a}$
SGR	$1.54±0.07^{a}$	$1.66±0.33^{a}$	$1.77±0.47^{a}$	$2.27±0.66^{a}$	$2.40±0.11^{a}$	$2.27±0.53^{a}$
Survival (%)	$36.67±5.77^{a}$	$43.33±5.77^{a}$	$76.67±5.77^{b}$	$86.67±5.77^{b}$	$80.00±10.0^{b}$	$86.67±5.77^{b}$
WBAA ($\mu g.g^{-1}$ tissue)	$2.27±0.18^{a}$	$3.86±0.25^{b}$	$7.61±0.14^{c}$	$8.82±0.34^{d}$	$10.74±0.09^{e}$	$12.5±0.11^{f}$

Means within the same row with a common superscript are not significantly different ($p>0.05$).

AAE·kg^{-1} diet when compared to the control diet and 50 mg AAE·kg^{-1} diet. Percentage weight gain was highest for prawns fed the supplemented diet using 250 mg AAE· kg^{-1} diet (224.39%) followed by 150 mg AAE·kg^{-1} diet (214.57%) and 500 mg AAE·kg^{-1} diet (211.12%). Lowest weight gain was observed for the diet devoid of vitamin C (112.62%). Specific growth rate also showed the same trend. At the end of the experiment, no statistically significant difference ($p>0.05$) in the specific growth rate (range = 1.54 to 2.40) of prawns fed with diets containing different levels of vitamin C was observed.

Weekly trend of mortality rate of prawns fed with diets containing graded levels of vitamin C are shown in figure 1. The mortality rate of prawns fed the control diet and the diet containing 50 mg AAE·kg^{-1} (ApP 0, ApP 50 diets) increased after 14 to 21 days. Mortality was observed to be associated with moulting and the dead animals had a soft-shell. Some prawns also showed symptoms of exuvia entrapment. After 28 days, the survival rate of prawns fed with diets containing 0 and 50 mg AAE·kg^{-1} diet was significantly lower ($p<0.05$) when compared to the others. Within the range of dietary vitamin C levels tested, the survival rate of prawns ranged from 36.7 to 86.6% as the dietary levels of vitamin C increased from 0 to 500 mg AAE· kg^{-1} diet. Vitamin C requirement was estimated by fitting a broken line model to the survival data and was found to be 135 mg AAE·kg^{-1} diet (Fig. 2). After seven weeks of the feeding trial, survival rate of prawns fed with diets containing 100, 150, 250 and 500 mg AAE·kg^{-1} diet did not show any statistically significant ($p>0.05$) difference.

The WBAA content of *M. rosenbergii* juveniles fed with different levels of dietary ascorbic acid is presented in table 3. Average WBAA content on the initial day of the experiment, following two weeks depletion was 3.98 ± 0.23 µg·g^{-1}. At the termination of the experiment, the WBAA content of the prawns fed with diets containing 0, 50 and 100 mg AAE·kg^{-1} diet were significantly lower ($p < 0.05$) than those of the prawns fed with diets containing 150, 250 and 500 mg AAE·kg^{-1} diet. WBAA content of prawns fed with the diet containing 0 mg AAE·kg^{-1} diet after 49 days of experiment was reduced from 3.98 ± 0.23 to 2.27 ± 0.18 µg·g^{-1}. The highest WBAA content was recorded for prawns fed with 500 mg AAE·kg^{-1} diet (12.5 ± 0.11 µg·g^{-1}), followed by 250 mg AAE· kg^{-1} diet (10.74 ± 0.09 µg·g^{-1}) and 150 mg AAE·kg^{-1} diet (8.82 ±0.34 µg·g^{-1}).

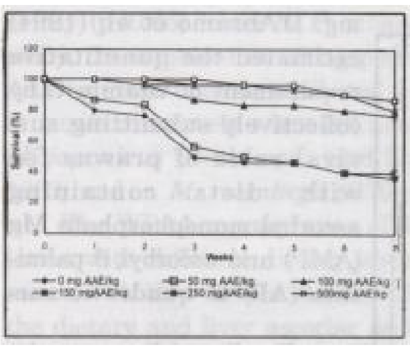

Fig. 1. Weekly survival of *M. rosenbergii* fed with graded levels of ascorbyl -2-polyphosphate.

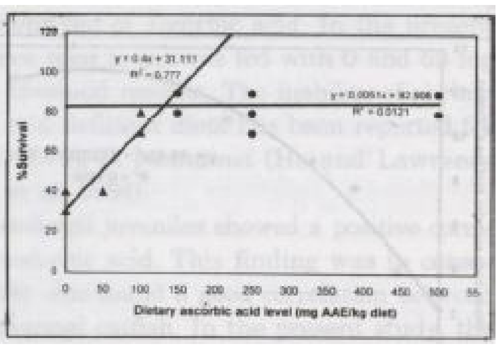

Fig. 2. Broken line model for the ascorbic acid requirement of *M. rosenbergii* juveniles. (Survival breakpoint is at 135 mg AAE·kg·diet^{-1}.)

The effect of different dietary vitamin C levels on the WBAA content of juveniles of *M. rosenbergii* is expressed as polynomial regression Y = -6E-05x^2 + 0.0523x + 2.1822, r^2 = 0.9766) and the trend is depicted in figure 3.

Discussion

Weight gain as well as improved survival rate recorded for the *M. rosenbergii* juveniles fed with diets containing ascorbyl 2 polyphosphate in the present study demonstrates that *M. rosenbergii* effectively utilizes ascorbyl 2 polyphosphate as a source of dietary vitamin C in prawn feeds. In *L. vannemei* the inclusion of ApP in diets resulted in the improvement of growth and survival (He and Lawrence 1993). Earlier studies showed that other ascorbic acid derivatives such as ascorbyl monophosphate and ascorbyl 6 palmitate are also successful as dietary sources of vitamin C for *M. rosenbergii* (D' Abramo et al. 1994).

Most of the vitamin C deficiency syndromes in penaeid shrimp such as 'Black Death' are related to the malfunction of collagen synthesis (Lightner et al. 1979) and vitamin C is essential for the synthesis of collagen. Impaired ecdysis, as observed in the present study, is similar to that seen in penaeid shrimp (He and Lawrence 1993) and in freshwater prawns (D' Abramo et al. 1994). High mortality occurred in *L. vannamei* fed with vitamin C deficient diet within two weeks (He and Lawrence 1993). *P. monodon* showed lower survival in none supplemented control diets compared to shrimp fed with diets supplemented with ascorbic acid. In the present study high mortality occurred between the third and fourth weeks of experiment and was mostly associated with moulting. Similar observations were made for penaeid shrimps (Lightner et al. 1979, He and Lawrence 1993) and for *M. rosenbergii* (D'Abramo et al. 1994). This lower survival rate indicates that *M. rosenbergii* require dietary vitamin C as reported by D'Abramo et al. (1994). Boonyaratpalin and Phongmaneerat (1995) and Reddy et al. (1999) found that *P. monodon* fed with ascorbic acid deficient diets showed poor feed intake, anorexia and prolonged deficiency that resulted in the blackening of gills and lesions in the abdominal region. The broken line model for the survival of the prawns in the present study suggests that 135 mg AAE·kg^{-1} diet is required for normal survival and growth of *M. rosenbergii* juveniles with an average initial body weight of 74 mg. D'Abramo et al. (1994) estimated the quantitative requirement of vitamin C by collectively submitting survival rates of prawns fed with diets containing ascorbyl monophosphate Mg (AMP) and ascorbyl 6 palmitate (AP) to quadratic sur-

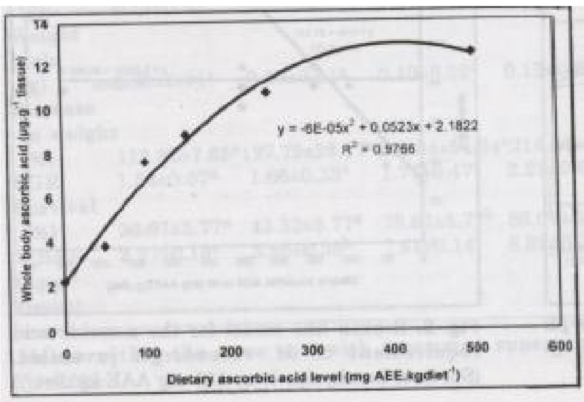

Fig. 3. The effect of dietary ascorbic acid on the WBAA content of *M. rosenbergii* juveniles.