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Effect of Coated Vitamin C on the Growth of *Macrobrachium rosenbergii* (de Man) Juveniles

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Experiment was conducted to study the effect of dietary vitamin C on growth and food conversion ratio of *Macrobrachium rosenbergii* juveniles, to determine the optimum dietary requirement of Vitamin C and to characterize the deficiency syndromes, if any. CVC F-90, a hydrogenated vegetable oil coated form of vitamin C, with 90% available ascorbic acid was used as the vitamin source. Growth and food conversion ratio were found to have significantly improved by incorporating dietary vitamin C. Deficiency syndromes like incomplete moulting and black lesions at the tip of walking legs were noticed below a level of 75 mg CVC F-90 / kg of dry diet. The maximum weight gain of 194.92% was obtained with a dietary level of 150 mg CVC F-90 / kg dry diet. Non-linear regression estimate based on percentage weight gain showed the optimum requirement to be 200.3 mg CVC F-90 / kg dry diet that is equivalent to 182.97 mg Ascorbic Acid Equivalent/kg dry diet.

Key words: ***Macrobrachium rosenbergii*, CVC F-90, vitamin C, ascorbic acid, deficiency syndrome.**

Introduction

Vitamins form an important group of micronutrients that is required by aquatic organisms for normal growth, metabolism and reproduction (Halver, 1989). Among the different vitamins required, vitamin C (ascorbic acid) has received much attention, probably due to the greater susceptibility of finfish and shellfish to its deficiency that leads to high mortality. Vitamin C is required for normal collagen synthesis and its deficiency leads to skeletal deformities such as scoliosis and lordosis in fish (Halver, 1989). In shrimp, it can result in poor growth and mortality associated with incomplete moulting (Lightner *et al.*, 1979; Magarelli *et al.*, 1979; Hunter *et al.*, 1979; He and Lawerence, 1993). Also the role of vitamin C as an immunostimulant in the shrimp diet was highlighted by Felix and Sivakumar (2003) and Lopez *et al.* (2003). *Macrobrachium rosenbergii* (de Man) is a commercially important species suitable for farming both in freshwater and low saline waters. It is widely cultured worldwide in tropical and temperate climates. The present study was conducted to determine the influence of Vitamin C on growth and food conversion ratio (FCR) of *M. rosenbergii* and to characterise any deficiency syndromes with a view to assess the optimum dietary requirement of Vitamin C for its normal growth and metabolism.

Materials and Methods

A coated form of vitamin C (CVC F-90 with 90% available vitamin C) manufactured and marketed by M/s Takeda Vitamin and food Asia PTE Ltd, Japan was used in the present study, since the source was much cheaper in terms of available ascorbic acid compared to the various ascorbic acid derivatives (Igarashi, 1994). Eight semi-purified and isoproteinacious diets (C_1 to C_8) were prepared (Sherief *et al.*, 1992) incorporating eight levels of vitamin C, in the forms of CVC- F-90 i.e. 0, 50, 75, 100, 125, 150, 200 and 500 mg/kg diet or 0, 45, 67.5, 90, 112.5, 135, 180 and 450 mg AAE (ascorbic acid equivalent)/kg diet respectively. The

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composition of the experimental diet is given in Table 1.

Healthy, well-pigmented juveniles of average initial length 51±4.5 mm and weight 61.24±7.43 mg produced from a single brood at the Freshwater Prawn Hatchery of the College of Fisheries, Kochi were selected for the experiment. The experiment was conducted for a period of 56 days using fiberglass tanks of 55cm diameter and 35 cm height with 75 L of water, each stocked with 10 animals. Eight treatments with 3 replications each were maintained. Prior to initiation of the feeding trial, the animals were acclimatized for one week on the conditioning diet containing no vitamin premix in order to deplete the vitamin resources in the tissue, to increase the level of response to the experimental diet and to familiarize the prawns to the experimental semi purified diets (He and Lawerence, 1993). Feeding was done twice daily during the first 4 weeks at the rate of 20% of the body weight, which was reduced to 10 % in the last four weeks. Every day before giving feed, the remnants of feed of the previous day were collected and dried at 60°C for calculating the actual feed consumed. Moulting and abnormal syndromes, if any, were recorded, and the excreta and exuvia were removed every day. About 75 % of water was changed daily and the tanks were scrubbed to prevent algal growth. The weight of dead specimens was also noted for computation of total weight gain. Growth assessment was done every fortnight and after a period of 56 days the prawns were captured for final assessment of calculating the percentage weight gain and specific growth rate.

The experiment was planned using completely randomised design and the data were analysed using analysis of variance (ANOVA) technique at 1% level of significance (Snedecor and Cochran, 1968). The optimum level of dietary vitamin C was determined from percentage weight gain using second order polynomial regression analysis, which describes the relation between dietary vitamin C level (x) and percentage weight gain (y) in curvilinear fashion ($y = a+bx+cx^2$).

Results and Discussion

The values obtained for the percentage weight gain and specific growth rate are given in Fig. 1 and 2 respectively. Both the

Table 1. Composition of Experimental Diet

Diet Ingredients (g)	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8
Casein (vitamin free)	45.0	45.0	45.0	45.0	45.0	45.0	45.0	45.0
Egg Albumin	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Glucose	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Sucrose	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Glucosamine HCl	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Acorn starch	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6
Sodium citrate	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sodium succinate	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Mineral Mix	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Carboxy methyl cellulose	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Cod Liver oil	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Maize oil	4.0	4.04.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mix (excluding vitamin c)	1.27	1.27	1.27	1.27	1.27	1.27	1.27	1.27
Vitamin C (CVC-90)	0	0.005	0.0075	0.01	0.0125	0.015	0.02	0.05
Cellulose (filler)	10.23	10.225	10.2225	10.22	10.217	10.215	10.21	10.18
Total	100	100	100	100	100	100	100	100

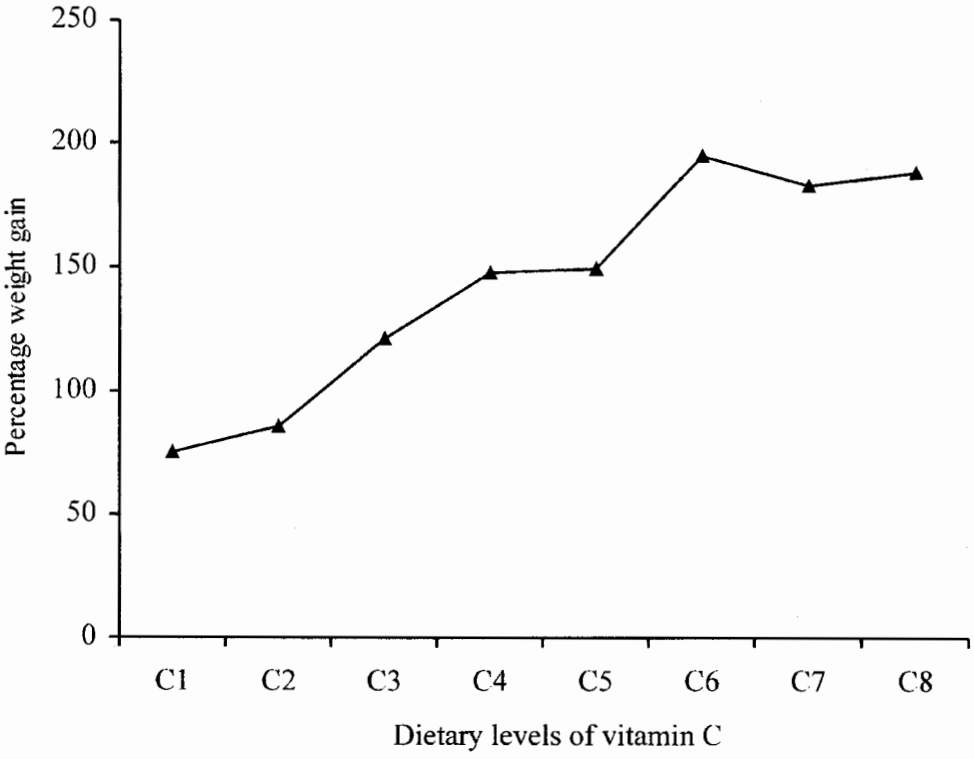

Fig. 1. Percentage weight gain of *Macrobrachium rosenbergii* fed different test diets

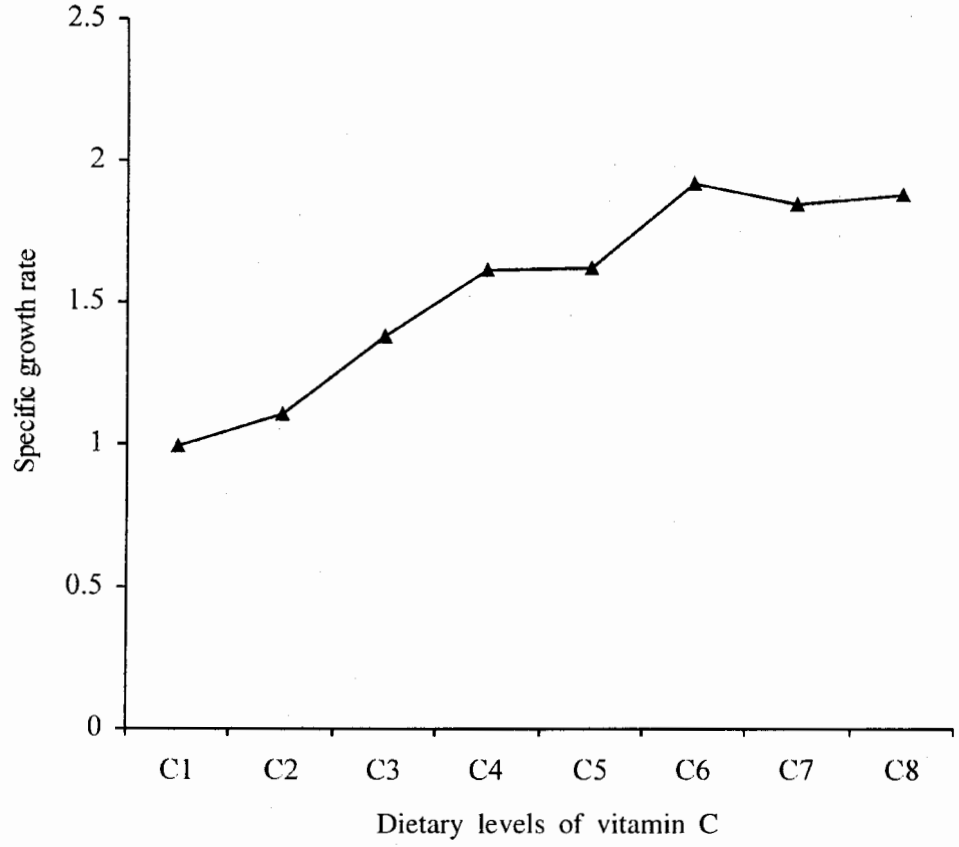

Fig. 2. Specific growth rate of *Macrobrachium rosenbergii* fed different test diets

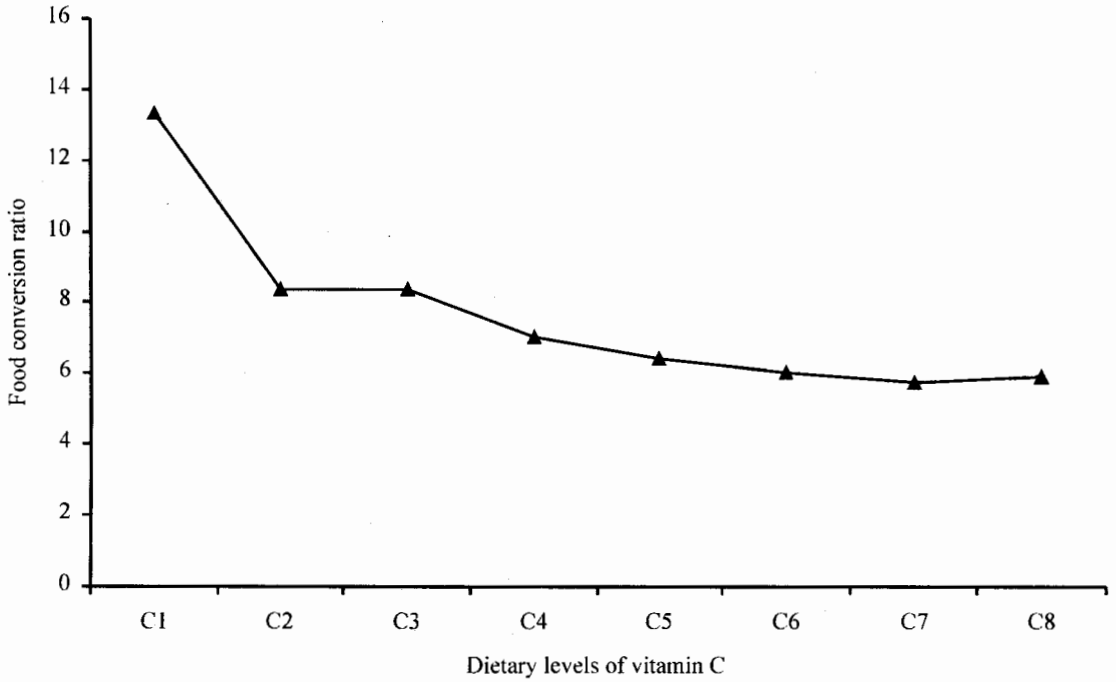

Fig. 3. Food conversion ratio of *Macrobrachium rosenbergii* juveniles fed different test diets

parameters showed similar changes. Analysis of variance showed that the influence of various dietary levels of vitamin C was significantly different ($p<0.01$). Pair wise comparison showed that the growth in treatments C6, C_7 and C_8 with dietary levels of 135, 180 and 450mg AAE / kg respectively was significantly high compared to treatments C_1, C_2, C_3, C4 and C_5. The highest value was obtained for treatment C_6 with 135 mg AAE/kg diet. The growth was slightly reduced at higher concentration of Vitamin C.

In *Marsupenaeus japonicus*, (Deshimaru and Kurokoi, 1976) and *Litopenaeus vannamei* (He and Lawerence, 1993) growth was not affected, when fed on a diet that contained no ascorbic acid. This might be because of the ability of the prawn to utilise certain micro algae as ascorbic acid source (Magarelli *et al.*, 1978: Baticoides *et al.*, 1992). Shrimps also have limited ability to synthesise ascorbic acid; while in young shrimp it is insufficient to meet the requirements, it is apparently sufficient to meet the requirement of larger shrimps of 12g size or more (He and Lawerence, 1993). But in *Penaeus monodon* the growth was significantly reduced when fed a Vitamin C deficient diet (Hsu and Shiau, 1998). In the present study also, there was a significant reduction in growth for ascorbic acid deficient diet. This might be because the experimental animals were smaller in size and steps taken to prevent the growth of algae in the experimental tanks. Similar results were obtained in *Litopenaeus stylirostris* and *Farfantepenaeus californiensis* (Magarelli *et al.*, 1979). D'Abramo *et al.*, 1994, had also shown that in *M. rosenbergii*, the growth rate was lower in groups fed with low vitamin C diet, although the differences were not significant.

A reduction of growth with high vitamin C is reported for *M. japonicus* (Deshimaru and Kuroki, 1976) and *Fenneropeneaus indicus* (Gopal, 1986). In the present study also growth reduced slightly at higher levels, showing that excess levels of vitamin C may retard the growth of *M. rosenbergii*.

The optimum dietary requirement of vitamin C was determined with respect to percentage weight gain, which is the prime

factor when commercial culture is considered. The estimation was done by fitting second order polynomial regression line ($y= 46.4597 + 1.2607 x - 0.0031 x^2$). The optimum value obtained was 203.3 mg CVC F-90 / kg diet or 182.97 mg AAE/kg diet. The optimum requirement for the same species obtained with phosphate and palmitate derivatives of ascorbic acid was 104.3mg AAE/kg diet (D' Abramo *et al.*, 1994). The greater requirement obtained in the present study might be due to lesser stability of coated form of ascorbic acid. Nevertheless, the availability is 90%, and thus the final amount of coated form of vitamin C required would be less compared to other derivatives.

FCR obtained for the different diets are given in Fig. 3. Analysis of variance of the data obtained showed that the FCR values differed significantly ($p<0.01$). The treatment C_1 with 0 level of vitamin C gave the highest FCR (13.36) showing very poor efficiency for food conversion. The lowest value for FCR (5.75) was given by diet C_7 (180mg AAE / kg diet). Above a dietary level of 90 AAE/ kg diet the FCR values were almost comparable. A higher FCR for lower level of dietary vitamin C was also obtained for *F. indicus* (Gopal, 1986) and *P. monodon* (Shiau and Jan, 1991). However, in *M. japonicus* it was observed that although the feed conversion efficiency remained constant for all treatments including 0 level vitamin C, the amount of food ingested by the prawn decreased as dietary ascorbic acid increased (Deshimaru and Kuroki, 1976).

The results of the present study showed that a dietary vitamin C level >90mg AAE/ kg diet was required to prevent deficiency symptoms. Black death, the typical vitamin C deficiency disease was observed in the first two treatments with 0 and 45 mg dietary vitamin C / kg diet. Below a level of 67.5mg vitamin / kg diet, the growth, survival and food conversion were very poor. The deficiency symptoms included black lesions at the tip of appendages of the prawns, and death due to incomplete moulting most of which occurred during the second fortnight. Black Death disease of ascorbic acid deficient shrimps has been studied by several workers (Lightner *et al.*, 1979; Magarelli *et al.*, 1979; Hunter *et al.*, 1979) who reported that within four weeks on an ascorbic acid deficient diet, black hemocytic lesions were observed in the loose connective tissues of the body, especially in those areas that are subjected to most mechanical trauma. Once the disease signs are observed, the process is irreversible and the affected animals will become anorexic and die within 24 to 72 hrs. These symptoms were observed in the present study also.

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