Original Research Article

International Journal of Bioassays
ISSN: 2278-778X
www.ijbio.com
OPEN ACCESS

INFLUENCE OF VITAMIN C ON GROWTH INDICES, SURVIVAL AND MOULTING RATES OF THE JUVENILES OF *MACROBRACHIUM ROSENBERGII*

Janardana Reddy S
Department of Fishery Science and Aquaculture, Sri Venkateswara University, Tirupati-517 502, India

Received for publication: April 22, 2014; **Revised:** May 17, 2014; **Accepted:** June 06, 2014

Abstract: The present work is aimed to find out the influence of dietary vitamin C on the survival, moulting rate and ascorbic acid content in tissues of juveniles of freshwater prawn, *Macrobrachium rosenbergii*. Prawns are treated with Vitamin C supplemented with a diet at eight levels of vitamin C *i.e.* 0, 50,100, 150, 200, 275, 375 and 525 mg AA (ascorbic acid)/kg (V0, V50, V100, V150, V200, V275, V375 and V525 respectively) to the experimental animals for 56 days. The results showed that below a level of 100 mg AA/kg diet, length of the animal, weight of the animal and the survival rates were deteriorated more whereas above the levels of V200 they are accelerated more, which was indicating mainly due to incomplete moulting. Black lesions and ulcerations at the tip of the appendages and on gills respectively are observed at the edge of the appendages of paralyzed and dead animals. The whole body ascorbic acid content reached a steady state only a 375 mg AA/kg diet and therefore the amount of ascorbic acid recommended in the diet of *M. rosenbergii* is 375 mg AA/ kg. It is evident from the results that vitamin C improves growth, survival and resistance in PLs of *M. rosenbergii* against stress during culture. Therefore, adequate supply of these vitamins in diet increases the commercial production of this species.

Key Words: Vitamin C, *Macrobrachium rosenbergii,* Survival rate, Moulting rate, Incomplete moulting

INTRODUCTION

It is well known that the aquaculture industry is in a phase of rapid development and growth, and the intensification of aqua farming often leads to the emergence of infectious and parasitic diseases. Few vaccines are commercially available in the market and their efficacy may be questioned in several cases, at least under practical field conditions. Aquaculture farming need the availability of chemotherapeutic agents to avoid severe economic losses, and chemotherapy will remain one of the main means of controlling transmissable diseases in the future [1].

Crustacean farming which are commercially important prawns and shrimps has made significant progress during the last few decades in many parts of the world due to luxuriant profitable commodity. The most favored and customer demanded species were those which commanded the highest prices when sold as luxury foods. Increasing public demand and benefits have provided sufficient stimulus to promote investment in prawn culture by the private sector. In order to help and solve the problems of the industry which have arisen due to rapid expansion and growth of their culture, studies are required on management techniques, to improve the quality and quantity of aqua products. [2, 3].

Macrobrachium rosenbergii is a commercially important species for diversifying aquaculture due to its attributes of becoming gravid in captivity, availability of established techniques of seed production in hatcheries and grow-out culture, wide consumer acceptance and high market value. Post larval stages of prawns are mainly benthic in nature, feed on detritus and other organic matter. In aqua culture practices number of formulated feeds has been used for post larvae which are available in the market. Formulated diets are alternatives because they exclude the need for costly live feeds. The formulated commercial diets are also available throughout the year, stored conveniently, easy to use and their nutrient composition is easily modified. In some cases, commercial products are not very rich in all nutritional qualities. Nutritive quality of larval feed is more important, as growth and quality of post larvae is highly correlated with quality seed production for aquaculture. The low quality seed may affect the final production of any farm and therefore the demand for such seed is reduced. Hence hatcheries should ensure the production of high quality seed [4, 5].

"It is true that the nutrients are identified by their ability to alleviate specific deficiency symptoms when fed to various mammals and birds. Although absolutely vital for various aspects of growth, health or reproduction, only little amounts are required in the diet. This phenomenon was explained by the idea that the role of these essential nutrients was limited to serving as co-factors for enzymatic reactions" [6].

***Corresponding Author:**
Dr. S. Janardana Reddy,
Department of Fishery Science and Aquaculture,
Sri Venkateswara University,
Tirupati-517 502, India.

Vitamin C is the generic descriptor for all compounds exhibiting qualitatively the biological activity of ascorbic acid. It plays an important role in many biochemical processes. Though an essential nutrient in most aquatic animals nutrition, it cannot be produced efficiently by the animals themselves. Vitamin C is essential for normal growth, immunity and reproduction in finfish and shellfish [7, 8].

The role of vitamin C in disease resistance has been studied in several fish. Durve and Lovell [9] found that a dietary supplementation of 30 mg vitamin C/kg supported normal growth and prevented deficiency signs in channel catfish. Increased resistance against infection by *Edwardsiella tarda* was observed at a supplementation level of 150 mg/kg at water temperature of 21°C; however, at 33°C, increasing the supplemented level of vitamin C had no significant effect on resistance against infection. Feeding channel catfish a megadose (3000 ppm) of vitamin C enhanced disease resistance against *E. ictaluri* significantly.

In prawn culture, 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 [10].

Several reports have been indicating that, in general, the vitamins improve immunity, meat quality, survival, growth rates, resistance against diseases and stressors, fecundity and reproductive efficiency. Vitamin C has also a good role in compatibility with unsuitable environmental conditions, bony structure, improvement of larvae quality in stressful conditions and enhancement of immunity system against pathogens [11, 12, 13].

Ascorbic acid (vitamin C) is also one of the most important micronutrients in both of fisn fish and shell fish caused to high mortality due to its deficiency [14]. Vitamin C is required for normal collagen synthesis and therefore its deficiency leads to mortality associated with incomplete moulting in crustaceans [15, 16, 17]. It also plays an important role in disease resistance [18]. It is highly significant in the present day aquaculture scenario bestowed with problems of disease and associated mortality. Deshimaru and Kuroki [7] made the first statement that the vitamin C deficiency in *Marsupenaeus japonicus* caused mortality or less survival of its post larvae was the most important parameter in his study. Moreover, it is also reported that the optimum dietary supplementation of vitamin C is equivalent to the amount that maintains a steady state or enhance the survivability of prawns [19].

The survival of prawn larvae was extremely low when they were fed on diets lacking vitamin C. Addition of vitamin C to a squid based diet for Juvenile *P. japonicus* accelerated growth rate, however, excess of vitamin C inhibited growth. Prawn grew best at inclusion levels of 0.22% [20, 21]. Iwata and Shigeno [22] reported the whitening induced by vitamin C deficient diets. Lightner *et al.,* [15] have found that *P. californiensis* and *P. stylirostyis* sometimes showed an abnormal symptom, named “Black Death” with a characteristic blackening of esophagus wall, cuticle, gastric wall, hind gut wall and gills. “Black death” has been recognized as a symptom of Vitamin C deficiency [16, 23, 24].

Since *Macrobrachium rosenbergii* is an important candidate species in aquaculture of both tropical and temperate climates, the present study was conducted with a view to determine the influence of dietary supplementation of vitamin C on survival, moulting rate and whole body ascorbic acid content in juvenile prawns.

MATERIALS AND METHODS

Source of Post Larvae

Healthy broodstock of *M. rosenbergii* was collected from the reared broodstock tanks (1500 L) of Sri Sai Venkateswara Giant Fresh Water Prawn Hatchery, Tupilipalem, Vakadu Mandal, Nellore District, India. Mature, berried females were transferred and maintained in aquamarine colored Fiber Reinforced Plastic (FRP) tanks of 500L capacity, having 12% salinity water. The larvae obtained from those mother prawns were reared in 300 L capacity larval rearing tanks and were initially fed with *Artemia* nauplii and later with egg custard, while gradually decreasing the salinity of water up to zero by the time the larvae reach the postlarval stage.

Preparation of Formulated Feed with Enrichments

The composition of the formulated feed used in the present study is presented in Table 1. Locally available dried ground shrimps, soybean meal and casein were used as protein sources. All dry ingredients except vitamin, mineral mixture and oil were mixed thoroughly to prepare the dough. Vitamin and mineral mixture was mixed with the oil and then with the dough. The dough was passed through a hand noodle maker and the feed strings were dried under low temperature, at approximately 40-50°C in an oven.

Dried feed strings were crushed into small particles manually and stored in the refrigerator for further use.

Preparation of Enrichment Solution of Vitamin C

Vitamin C coated with vegetable oil having 90% available ascorbic acid, known as Vitamin C (L–Ascorbyl 2 monophosphate-AMP) was procured from E-MERCK Darmstadt, Germany, used as the vitamin C source in the present study. Compared to phosphate and sulphate derivates of ascorbic acid, this compound is reported to have better performance with respect to available ascorbic acid per unit weight and cost. Eight semi-purified, isoproteinaceous diets were prepared [25] incorporating eight levels of vitamin C *i.e.* 0, 50,100, 150, 200, 275, 375 and 525 mg AA/kg diet. In order to study the effect of excess and deficient dietary inclusion of ascorbic acid, a wide range from 0 to 400mg AA/ kg diet was selected. Since the range of optimum dietary requirement was believed to be between 45 and 135mg AA/kg diet based on previous works [17, 24], short intervals were given between these dosages. The vitamin supplemented diets are represented as V_0, V_{50}, V_{100}, V_{150}, V_{200}, V_{275}, V_{375} and V_{525} respectively. Proximate analysis was done as per AOAC [26] methods after the feed preparation (Table 1).

The experiment was conducted using healthy, well-pigmented juveniles of average weight 62.19 ± 4035 mg with initial length of 54.26 ± 3.96mm, produced from a single brood at the Freshwater Prawn Hatchery. Fiberglass tanks of 75 cm diameter and 50 cm height filled with 100 liters of water were used with 10 animals each in triplicates. Feed was given at a rate of 20% of the body weight during the total experimental period. Mortality and abnormal syndromes, if any, were recorded every day and discarded from the stocking ponds. About 75 % of water was exchanged and exuvia, faecal matter and uneaten feed were removed daily and the tanks were scrubbed to prevent algal growth. Water quality parameters were checked at weekly intervals and their ranges are recorded (Table 2). The experiment was conducted for a period of 65 days. After expiry of each experimental period, prawns in each treatment were pooled together and also samples are collected and also pooled together from each exposure group for analyses of whole body ascorbic acid [27]. The survival rate and moulting rates were calculated by using the following formula of Petriella [28].

$$\textbf{Survival rate} = \frac{\text{Final number of prawn} \times 100}{\text{Initial number of prawn}}$$

$$\textbf{Moulting rate} = \frac{\text{Moult percentage}}{\text{Mean life of group}}$$

Where: **Moult percentage** = Number of moults x 100/ initial number of animals.

Prawns Performance

Prawn post larvae from each individual tank were randomly selected and dried with filter paper and then weighed in digital electronic balance up to 0.01g. Growth parameters were calculated by using the formulae of Turchini *et al.*, [29].

1. **Weight Gain (WG) = W2 - W1**

Where: W_1 and W_2 are the initial and final weight (g)

2. **Specific Growth Rate (SGR)= ln W_2 - W_1 / t × 100**

Where: W_1 and W_2 are the initial and final weight (g), respectively, and t is the number of days in the feeding period.

3. **Daily Growth Index (DGI)=(Wt–W0)×100/t**

Where: W_t and W_0 were final and initial fish weights, respectively.

4. **Condition Factor (CF)=W/L3×100**

Where: W is final weight (g), L is total length (cm).

Statistical analysis

Results are presented as means ± standard error of means (SEM). Differences among the control and treatment means were analyzed by one-way analysis of variance (One way ANOVA) followed by Duncan's new multiple range tests. Differences were considered statistically significant when $P < 0.05$. Statistical analyses were carried out using the SPSS statistical package (SPSS Inc., Chicago, IL, USA).

RESULTS

The influence of various levels of dietary supplemented ascorbic acid on juveniles of *Macrobrachium rosenbergii* has been determined with reference to survival rate, moulting rate and whole body ascorbic acid content. Water samples from the experimental tanks were also analyzed for dissolved oxygen and total ammonia nitrogen at weekly intervals. Temperature and pH were monitored daily using a mercury thermometer and pH meter respectively (Table 1). Proximate compositions of the experimental feed used at various levels of supplemented Vitamin C diets are presented in Tables 2, 3. 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 4. A significantly higher ($p < 0.05$) increase in length was recorded in diets supplemented with vitamin C levels of 200 mg AA/kg-1 diet when compared to the control diet and 50 mg AA/kg^{-1} diet.

The maximum length, weight and survival rate were observed at the level of 525 mg AA/kg^{-1} diet. The WBAA content of *M. rosenbergii* juveniles fed with different levels of dietary ascorbic acid is presented in table 4. The highest WBAA content was recorded for prawns fed with 525 mg AA/kg-1 diet (16.49 ± 0.18 µg·g-1), followed by 375 mg AA/kg-1 diet (14.56 ± 0.16 µg·g-1) and 275 mg AA/kg-1 diet (13.85 +0.85 µg·g-1).

Table 1: Range of water quality parameters maintained in the experimental fish tanks

S.NO	Water Quality Parameters	Range of Parameters
1	Dissolved Oxygen	6.6 - 7.3 ppm
2	Ammonia-N	0.02 - 0.04 ppm
3	Nitrite-N	0.02 - 0.07 ppm
4	Total alkalinity	90.3 - 96.3 mg/l as Ca CO3
5	Total hardness	106.3 - 116.3 mg/l as aCO3
6	pH	7 - 8
7	Temperature	27.9 - 28.2°C

§ Composition of vitamin–mineral mixture (quantity/ kg)

Vitamin A 6,25,000 IU; Vitamin D3 62,500 IU; Vitamin E 250 mg; Nicotinamide 1 g; Cu 312 mg; Mg 6g; Fe 1.5 g; Zn 2.13 g; I 156 mg; Se 10 mg; Mn 1.2 g; Ca 247.34 g; P 114.68 g; S 12.2 g; Na 5.8 mg; K 48.05 mg.

Composition of vitamin B complex (quantity/ g)

Thiamin mononitrate 20 mg; Ribloflavin 20mg; Pyridoxin hydrochloride 6 mg; vitamin B12 30µg; Niacinamide 200mg; Ca pantothenate 100mg; Folic acid 3 mg; Biotin 200µg.

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

Proximate Composition	Different Experimental Diets							
	V_0	V_{50}	V_{100}	V_{150}	V_{200}	V_{275}	V_{375}	V_{525}
Protein	48.76	47.35	47.45	47.75	47.96	47.39	46.43	45.55
Carbohydrate	23.55	24.45	24.85	25.79	27.56	28.44	28.95	29.65
Lipid	8.34	7.86	7.35	7.05	6.83	6.68	6.54	6.15
Fibre	6.19	5.85	5.44	5.15	4.86	4.65	4.48	4.27
Ash	5.12	5.04	4.94	4.82	4.64	4.53	4.40	4.25
Moisture	10.53	10.24	10.16	9.83	9.10	8.67	8.43	8.29

Table 3: Composition of the formulated feed used in this study.

Ingredients	g kg^{-1}
Shrimp meal	300
Soybean meal	120
Casein	200
Starch	80
Cooking oil (sun flower)	100
Dextrin	50
Gelatin	50
Cellulose powder	50
Carboxy Methyl Cellulose	20
Vitamin- Mineral mixture	28
Vitamin B complex	02
Total	1000

Table 4: Weight, Length, SGR, Survival Rate and Whole Body Ascorbic Acid of juveniles *M. rosenberger* fed with graded levels of Vitamin C for 62 days.

Parameter	V_0	V_{25}	V_{50}	V_{100}	V_{150}	V_{200}	V_{275}	V_{375}	V_{525}
Initial Length (cm)	2.69±0.12	2.89±0.03	3.12±0.04	3.23±0.02	3.19±0.12	3.29±0.14	3.48±0.16	3.65±0.15	3.84±0.17
Final Length (cm)	3.16±0.052	3.41±0.53	3.76±0.44	3.98±0.36	4.04±0.13	4.27±0.15	4.64±0.36	4.94±0.43	5.33±0.39
Increase in length (cm)	0.47±0.08	0.52±0.04	0.64±0.14	0.75±0.11	0.85±0.14	0.98±0.04	1.16±0.04	1.29±0.04	1.49±0.04
Increase in length (%)	18.24±3.10	19.54±2.16	21.49±7.51	24.51±5.88	30.20±6.55	31.92 ±3.22	34.16 ±3.22	36.95 ±3.22	39.48 ±3.22
Initial Weight (g)	0.07±0.01	0.07±0.01	0.07±0.02	0.08±0.02	0.09±0.01	0.11±0.02	0.12±0.02	0.12±0.02	0.13±0.02
Final Weight (g)	0.11±0.02	0.14±0.01	0.16±0.01	0.22±0.02	0.26±0.02	0.32±0.02	0.35±0.02	0.49±0.02	0.68±0.02
Weight Gain (g)	0.04±0.01	0.06±0.01	0.09±0.02	0.14±0.04	0.17±0.01	0.21±0.03	0.23±0.04	0.37±0.06	0.55±0.05
Increase in weight (%)	57.62 ±7.65	85.79 ±38.17	128.34±54.04	175.57±33.14	188.39±17.48	192.68±35.16	196.53±35.16	316.57±35.16	434.19±35.16
SGR	1.54±0.07	1.66±0.33	1.77±0.47	2.27±0.66	2.40±0.11	2.58±0.53	2.69±0.49	2.85±0.72	3.59±0.55
Survival (%)	36.67±3.65	43.33±3.51	76.67±5.83	80.65±5.77	84.00±4.08	86.58±5.48	89.62±6.74	92.61±5.73	96.49±5.37
WBAA (µg.g-1 tissue)	2.27±0.18	3.86±0.25	7.61±0.14	8.82±0.34	10.74±0.09	12.54±0.11	13.85±0.13	14.56±0.16	16.49±0.18

Values are significant at P< 0.05, Values are mean ± SD of six individual observations.

The survival percentage rates of juveniles obtained for the different supplemented dietary levels of vitamin C is represented in Figure 1. It is obvious from the results that the supplemented dietary vitamin C influenced on survival rate of juvenile prawns significantly and the difference (P<0.01). The supplemented diets V_{25} and V_{50} caused significantly lower survival rates (20% to 34%), whereas the survival rates with dietary levels V_{200}, V_{275} and V_{375} (79% to 92%) and the maximum mean survival rate (98.66%) was observed at the treatment of $V_{525}$with ascorbic acid level of 525 mg AA/kg diet. In all exposure periods, mortality occurred within 20days after initiation of the experiment, afterwards the rate of mortality gradually and significantly decreased (Figure 1). It is observed that the mortality in juveniles was associated with incomplete moulting, black lesions and ulcerations were observed at the tip of the appendages and on gills respectively of the dead animals. Hari and Kurup [30] during the study of Vitamin C requirement of the giant freshwater prawn *Macrobrachuim rosenbergii,* find out that the 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 AA/kg 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 AA/ kg diet (Figure 1).

Figure 1: Variations in Percent Survival rate of *Macrobrachium rosenbergii* fed with different levels of supplemented diet.

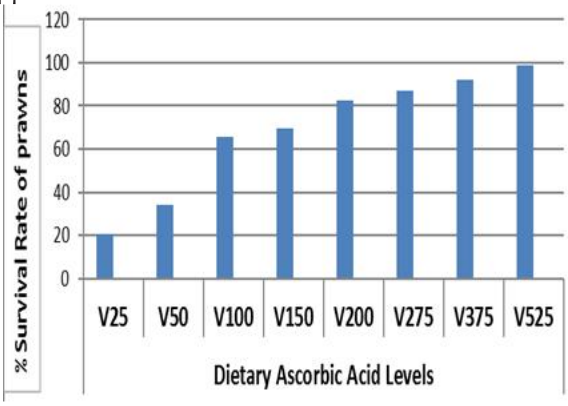


Figure 2: Variations in Percent Moulting rate of *Macrobrachium rosenbergii* fed with different levels of supplemented diet.

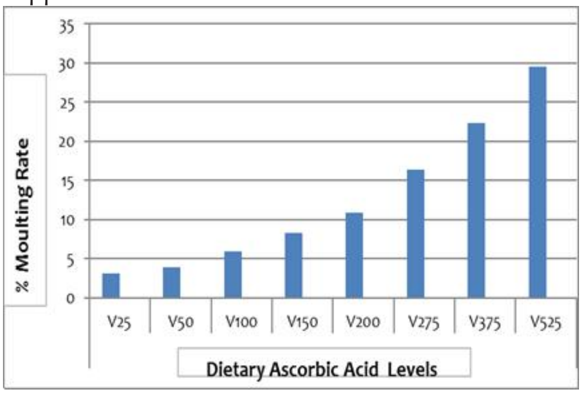
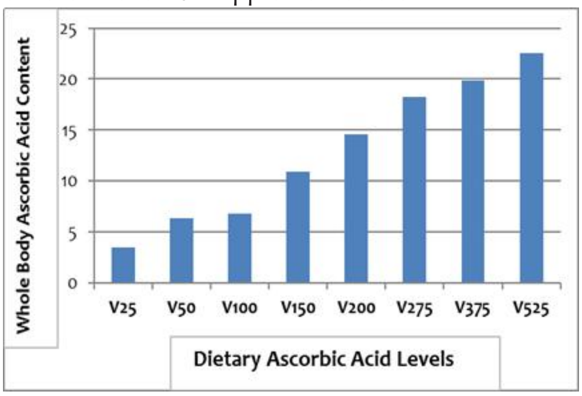


Figure 3: Variations in Percent Whole Body Ascorbic Acid Content of *Macrobrachium rosenbergii* fed with different levels of supplemented diet.



The moulting of prawns during the experimental period at various supplemented dietary levels of vitamin C showed significant variations (Figure-2). The analysis of variance of the moulting frequency demonstrated that the significant variations among the various supplemented dietary levels of vitamin C. It is evident that the dietary level of $\mathbf{V_{100}}$ and below this level moulting rate is significantly lower (3% to 5%). In the supplementary vitamin C dietary levels of V_{275} to V_{525} the rate of moulting was drastically increased up to 29% (Figure 2). The whole body ascorbic acid content of *M, rosenbergii* was determined at the end of each exposure period It is evident from the results that the total ascorbic acid content was gradually and significantly increased with increase of exposure period and the maximum increase (21.37%) was observed at the level of V_{525} (Figure 3).

DISCUSSION

It is evident from the results that the survival rate of juveniles of *M. rosenbergii* is significantly influenced by dietary supplemented vitamin C content and the rate of survival is gradually and significantly increased with increasing the exposure period. The maximum survival rate was observed at V_{525} (525 mg AA/kg diet) supplemented dietary level (Figure 1). The supplemented dietary Vitamin C was also caused to mortality of fish at all levels, however the rate of mortality is gradually decreased (Figure 2). It is obvious from various reports that survival is the prime factor affected by ascorbic acid deficiency, rather than growth.

At a supplemented dietary dietary level below 1000mg of vitamin C/kg diet, high mortality was reported in *M. japonicas* [7], *Litopenaeus stylirostris* and *Farfantepenaeus californiensis* [8]. L-ascorbyl-2-phosphate which was used as vitamin C source, at a dietary level below 100 mg AA/kg diet caused very poor survival rate in *Litopenaeus vannamei* [17, 31]. D'abramo /$/4/Ũ②/[24] reported that below a level of 50 mg AA/kg diet high mortality occurred in *M. rosenbergii* when ascorbyl–2 monophosphate calcium salt and ascorbyl - 6–palmitate were used as the vitamin C source.

The present study perceptible that a dietary level of 200 mg AA/kg diet is required to obtain > 89% survival of the test fish. High mortality rate was recorded at the early phase of the experiment and this may be imputed to the decreased rate of biosynthesis of vitamin C at early stage. Later on with increase in growth, mortality was slowly being reduced, which may be due to lesser requirement for the dietary vitamin C, as a result of its sufficient biosynthesis is occurred in the body [17]. Moulting rate was also observed to be significantly affected by the deficiency of dietary vitamin C. Prawns fed 25 mg AAE/kg diet had less number of moults, and mortality was mainly associated with incomplete moulting. High moulting rate was observed to be equal to normal moulting rate, when prawns fed with vitamin C at the rate of 275 - 525 mg/kg diet. Lightner *et al.,* [15] have reported the inability of ascorbic acid deficient shrimp to hydroxylate sufficient procollagen to produce mature collagen fibers. This may be tone of the reasons for poor moulting in prawns. The inability of ascorbic acid deficient shrimp to moult completely has been reported in *M. japonicas* [7], *Fenneropenaeus indicus* [32] and in *L. vannamei* [17]. Similar phenomenon was also also similar phenomenon has also been reported in *M. rosenbergii by* Heinen [3] and D' Abramo *et al.,* [24]

The whole body ascorbic acid content of the experimental prawns in the present study seemed to be directly related to supplemented dietary level of vitamin C. The tissue ascorbic acid content was not found to be dissipated in prawns even after feeding with a diet free of vitamin C for 62 days. It indicates that the ability of prawns to synthesize vitamin C sufficiently to some extent [15, 24, 33, 34]. It is evident from the results that the survival rate is increased with increase of total ascorbic acid content and the maximum survival (98.67%) was observed at the rate of >22µg/kg body weight of AA in the experimental prawns of 0.25g size. The studies on *Litopenaeus stylirostris* and *Farfantepenaeus californiensis* of 0.7g size disclosed that tissue levels >40µg AA/g is required for normal survival, while <25 µg AA/g tissue predisposed the animals to deficiency diseases [15]. Boonyaratpalin and Phongmaneerat [35] and Reddy *et al.,* [36] 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. Shiau and Jan [37] have verdict that for *Penaeus monodon* of 0.4 g size, the tissue levels >23µg AA/g is required for maximum survival. Thus it can be conclude that the tissue level of ascorbic acid for normal survival vary with species and size of shrimp. In the present study high mortality occurred between the second and third weeks of experiment prawns fed with lower doses of ascorbic acid and was mostly associated with moulting. Similar observations were made for penaeid shrimps [8, 17, 38] and for *M. rosenbergii* [24]. This lower survival rate indicates that *M. rosenbergii* more required dietary vitamin C as reported by D'Abramo *et al.,* [24]. Dabrouski [19] studied on the effect of dietary ascorbic acid supplementation on the accumulation of vitamin C in tissue in *Coregonus laveratus*, has suggested that the optimum dietary concentration of vitamin C is equivalent to the quantity required to maintain a steady state of tissue vitamin C in larvae and juveniles of fish. In the present study it is observed that there is a significant and gradual increase in the whole body ascorbic acid content of animals fed with 100, 150, 200, 275, 375 and 525 mg AA/kg supplemented diet, however at the V375 dietary level of vitamin C observed a constant elevation, so it seems to be 375 mg AA/kg diet is required by *M. rosenbergii* since the test prawns showed good survival rate and moulting rate at this concentration of supplemented dietary vitamin C.

Generally, vitamins play important roles in animal health as antioxidants by inactivating damaging free radicals produced through normal cellular activity and from various stressors [39, 40]. It has been suggested that the antioxidant function of these micronutrients could enhance immunity by preserving the functional and structural integrity of important immune cells. Vitamin C has good roles in compatibility with unsuitable environmental conditions, bony structure, improvement of larvae quality in stressful conditions and enhancement of immunity system against pathogens [41, 13]. It has been cleared that vitamin C is required by all animals for body maintenance, growth and other biological performances and the vitamin C level needed for these functions varies with the species and culture environment [42, 33]. In conclusion, our study showed that the vitamins especially vitamin C improves growth, survival and resistance in PLs of *M. rosenbergii* against stress during culture. Therefore, adequate supply of these vitamins in diet increases the commercial production of this species.

REFERENCES

1. Conklin DE, vitamin requirements of juvenile Penaeid shrimp. Advances in Tropical Aquaculture, 1989, 9, 287-308.
2. Mukhopadhyay PK, Rangacharyulu PV, Mitra G, Jana BB, Applied nutrition in fresh water prawn, *Macrobrachium rosenbergii* culture, J App Aquacult, 2003, 13, 317-340.
3. Heinen JM, Vitamin requirements of freshwater prawn *Macrobrachium rosenbergii*, J World Aquacult Soc, 1988, 19 (1) 36(A), 116.
4. Patricia MM, JR Tomasso, TM Brandt, Effects of Dietary Vitamin C on Growth, Caudal Fin Development, and Tolerance of Aquaculture-Related Stressors in Channel Catfish, The Progressive Fish-Culturist, 1987,49(1), 13-16.
5. Hunter B, PC Magarelli, Jr DV Lightner, LB Colvin, Ascorbic acid dependent collagen formation in Penaeid shrimp, *Comp Biocehm Physiol*, 1979, 64B, 381-385.
6. Guillaume J. Generalites. In: Guillaume J, Kaushik S, Bergot P, Metailler R. (Eds.) Nutrition et Alimentation des poissons et crustaces, INRA ed. Brest, France, 1999, 25– 39.
7. Deshimaru O, K Kuroki, Studies on a purified diet for prawn, VII. Adequate dietary levels of ascorbic acid and inositol Bull Jpn Soc Sci Fish, 1976. 42, 571-576.
8. Lightner DV, B Himtea, PC Magarelli Jr, LB Colvin, Ascorbic acid: Nutritional requirement and role in wound repair in penaeid shrimp, *Proc World Maricult Soc*, 1979, 10, 513-528.

9. Durve VS and Lovell RT, Vitamin C and disease resistance in channel catfish (*Ictalurus punctatus*), Can J Fish Aquat Sci, 1982, 39, 948-951.

10. Arup Tewary, Bidhan C Patra, Use of vitamin C as an immunostimulant, Effect on growth, nutritional quality, and immune response of *Labeo rohita* (Ham.), Fish Physiology and Biochemistry (Impact Factor: 1.55), 2008, 34(3), 251-9.

11. Guary M, Kanazawa A, Tanka N, Ceccaldi HJ, Nutritional requirements of prawn-IV Requirement for ascorbic acid, Memoris of the Faculty of Fisheries, Kangoshima University, 1976, 25, 53-57.

12. Conklin DE, Vitamin requirements of juvenile Penaeid shrimp, Advances in Tropical Aquaculture, 1989, 9: 287-308.

13. Racotta IS, Palacios E, Hernandez-Herrera R, Bonilla A, Perez-Rostro CI, Ramirez JL, Criteria for assessing larval and Postlarval quality of pacific white shrimp (*Litopenaeus vannamei* Boone), Aquaculture, 2004, 233, 181-195.

14. Halver JE, The vitamins, In *Fish nutrition* 2nd edition, Halver JE (Ed) Academic Press Inc, New York, 1989, 31-109

15. Lightner DV, Colvin LB, Brand C, Donald DA, "Black Death" a disease syndrome of penaeid shrimp related to a dietary deficiency of ascorbic acid, Proc World Maricul Soc, 1977, 8, 611-618.

16. Magarelli PC jr, B Hunter, DV Lightner, LB Colvin, Black death: an ascorbic acid deficiency disease in Penaeid shrimp, Comp Biochem Physiol, 1979, 63A, 103-108.

17. He H, AL Lawrence, Vitamin C requirement of the shrimp *Penaeus vannamei*, Aquaculture, 1993, 114 a, 305-316.

18. Felix S, K Sivakumar, 'Pro-p assay' to measure immune enhancement in *Penaeus monodon* (Fabricius). J Aquacult Trop, 2003, 18: 111-119.

19. Dabrouski K, Ascorbic acid status in the early life of white fish (*Coregonus laveratus*. L) Aquaculture, 1990, 84, 61-70.

20. Kitabayashi K, Shudo K, Nakamura K, Ishikawa S, Studies on formula feed for Kuruma prawn, On the utilization values of glucose, Bull Japan Soci Sci Fish, 1971b, 65, 109-118.

21. Harrison KE, The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans a review, Journal of Shellfish Research, 1990, 9, 1–28.

22. Iwata M, Shigeno K, Vitamin-C deficiency symptoms of prawn, *Penaeus japonicus*, Meeting of the Japanese Soci Sci Fish, Abstract, 1980, 8.

23. Shiau SY, FL Jan, Ascorbic acid requirement of grass shrimp *Penaeus monodon, Nippon Suisan Gakkaishi*, 1992, 58, 363.

24. Louis R, D' Abramo, Cynthia A, Moncreiff, Felix P, Holcomb, J Labrenty Montanez and Randal K. Buddington (1994) Vitamin C requirement of the juvenile freshwater prawn, Macrobrachium rosenbergii. Aquaculture, 128 (3-4): 269-275.

25. Sherief PM, CM Nair, V Mallika, The cholesterol requirement of larval and postlarval prawn (*Macrobrachium rosenbergii*), In Fresh water prawns, EG Silas (Ed), 1992, 213-217.

26. AOAC, Official methods of analysis Association of Analytical Chemists, Inc, Arlington USA, 1990.

27. Omaye ST, JD Tumbull, HE Sauberlich, Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids, In *Methods in Enzymology* Vol 62, (DB Mc Cormick and LD Wright (Eds) Academic press, Inc, Newyork, 1979, 3-11.

28. Petriella, AM, Study of the moulting cycle of the argentine prawn, *Artemesia longinaris*, Bate III Influence of cholesterol, J Aquacult Trop, 1990, 6, 77-86.

29. Turchini GM, Mentasti T, Froyland L, Orban E, Caprino F, Moretti VM, Valfrè F, Effects of alternative dietary lipid sources on performance, tissue chemical composition, mitochondrial fatty acid oxidation capabilities and sensory characteristics in brown trout (*Salmo trutta L)* Aquaculture, 2003, 225, 251-267.

30. Hari B, BM Kurup, Vitamin C (Ascorbyl 2 Polyphosphate) Requirement of Freshwater Prawn *Macrobrachium rosenbergii* (deMan), Asian Fisheries Science, 2002, 15, 145-154.

31. Lopez N, G Cuzon, G Gaxiola, G Taboada, M Valenzuela, C.Pascual, A Sanchez, P Rosas, Physiological, nutritional,and immunological role of dietary beta 1-3 glucan and ascorbic acid 2-monophosphate in *Litopenaeus vannamei* juveniles, *Aquaculture,*2003, 224, 223-243.

32. Gopal C, Nutritional studies on juvenile Penaeus indicus with reference to protein and vitamin requirement, Cochin University of Science and Technology, Cochin, India, 1986.

33. Lovell RT, Protein requirement of cage cultured channel catfish, Proceedings, Southeast Asian Association of game and fisheries commission, 1972, 26, 357-361.

34. Patricia M Mazik, JR Tomasso, TM Brandt, Effects of Dietary Vitamin C on Growth, Caudal Fin Development, and Tolerance of Aquaculture-Related Stressors in Channel Catfish, The Progressive Fish-Culturist, 1987, 49, 1, 13-16.

35. Boonyaratpalin M, Pongmaneerat J, Ascorbic acid derivative requirement of *Penaeus monodon,* Phuket Mar Biol Cent Res Bull, 1995, 60, 65-73.

36. Reddy HRV, MG Naik and TS Annappaswamy, Evaluation of the essentiality of vitamins for *Penaeus monodon,* Aquaculture Nutrition 5, 4, 1999a, 267-275.

37. Shiau SY, FL Jan Ascorbic acid requirement of grass shrimp *Penaeus monodon, Nippon Suisan Gakkaishi, 1992*, 58, 363.

38. New MB, A review of dietary studies with shrimp and prawns, Aquaculture, 1976, 9, 101-144.

39. Chew BP, Antioxidant vitamins affect food animal immunity and health, Journal of Nutrition, 1995, 125, 1804S-1808S.

40. FAO, The state of World fisheries and aquaculture food and agriculture Organization of the United Nation, Rome, 2006,

41. Gapasin RSJ, Bombeo R, Lavens P, Sorgeloos P, Nelis H, Enrichment of live food with essential fatty acids and vitamin C effects on milkfish (*Chanos chanos*) Larval performance, Aquaculture, 1998, 162, 269-286.

42. Anh NTN, Hien TTT, Mathieu W, Hoa NV, Sorgeloos P, Effect of fishmeal replacement with Artemia biomass as a protein source in practical diets for the giant freshwater prawn *Macrobrachium rosenbergii*, Aqua Res, 2009, 40, 669-680.

Source of support: Nil
Conflict of interest: None Declared