VITAMIN C REQUREMENT AND ITS DEFICIENCY SYNDROMES IN *MACROBRACHIUM ROSENBERGII* JUVENILES

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DEDICATED TO MY PARENTS

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1. INTRODUCTION

In recent years, aquaculture, especially the culture of shrimps and prawns, has received much attention in view of the increasing demand and price for these products in the international market. A number of species of penaeid prawns are cultivated in brackish water and marine environments. Among the fresh water species, the giant fresh water prawn, *Macrobrachium rosenbergii* is the most important one in view of its culture potential. The species has contributed to 3.2 % of the total cultured crustaceans in the world in 1992 (New, 1995).

For the culture of any organism in captivity, adequate feeding with a well-balanced diet is essential (Cho *et al.*, 1985). A well-accepted balanced diet is highly essential to overcome the various environmental stresses and to hasten early recovery from diseases. For the formulation of such a diet, adequate research is to be carried out to determine the optimum requirement for the species concerned of various nutrients such as proteins, lipids, etc. Works on the requirements of micronutrients such as vitamins and minerals are scarce.

Vitamins form an important group of micronutrients which is required by aquatic organisms for normal growth, metabolism and reproduction (Halver, 1989). Among the different vitamins required, vitamin C or Ascorbic acid has received much attention, may be because of greater susceptibility of fin fishes and shell fishes to its deficiency which leads to high mortality. Vitamin C is required for normal collagen synthesis and so its deficiency leads to skeletal deformities such as scoliosis and lordosis in fishes (Halver, 1989). In shrimps it can lead to poor growth and mortality associated with incomplete moulting (Lightner *et al.*, 1979 ; Magarelli *et al.*, 1979; Hunter *et al.*, 1979; He and Lawrence, 1993).

For *M. rosenbergii,* although vitamin C requirement has been studied by D' Abramo *et al.* (1994) using phosphate and palmitate derivatives of ascorbic acid, he himself has suggested

the use of cheaper sources of vitamin C for profitability in commercial culture. So in the present study a coated form of vitamin C (with 90% available vitamin C) known as CVC- F-90 was used, since the source is much cheaper in terms of available ascorbic acid compared to the various ascorbic acid derivatives (Igarashi, 1994). An experiment was conducted to determine the influence of dietary vitamin C on growth, survival, food conversion ratio, moulting rate and whole body ascorbic acid content in juvenile prawns, to assess the optimum dietary requirement of vitamin C and to characterise deficiency syndromes, if any.

2. REVIEW OF LITERATURE

2.1 Vitamin C nutrition in fishes and crustaceans

Crystalline vitamin C was first isolated and demonstrated as, antiscorbutic vitamin by King and Waugh in the year 1930 and was named as vitamin C by Drummond (Halver, 1972). Since then, a lot of research has been done to study its function in mammals and poultry. However relatively less work has been done in aquatic species, mainly due to inherent problems caused by aquatic medium such as leaching of the vitamin (Paulraj, 1987) and unstable nature of vitamin C in aquatic feeds (Hilton *et al.*, 1977; Shiau and Hsu, 1993). In the last two decades considerable research has been done on vitamin C requirement and related aspects in a number of aquatic species, especially of fishes and shrimps. A brief review of these studies is given in the following sections.

2.2. Bio-synthesis of vitamin C by aquatic species

Halver (1972), a pioneer of vitamin studies in aquatic species, was of the opinion that the fishes do not have the ability to synthesise vitamin C or ascorbic acid since they cannot produce L-gulonolactone oxidase enzyme which catalyses the conversion of L-gulonolactone to L-ascorbic acid. The synthesis of ascorbic acid was studied later by Dabrowski (1994) in two primitive actinopterygian fishes, the paddle fish (*Poliodon spathula*) and white sturgeon (*Acipenser trasmontanus*) by feeding with a scorbutogenic diet. He suggested that the modern bony fishes, Teleostei, lost this ability to express the gulonolactone oxidase genes after they had separated during the Silurian from the common ancestor with Coelacanths (*Latimaria*) and Dipnoi. Sato *et al.* (1978) reported that the common carp (*Cyprinus carpio*) do not require dietary vitamin C for growth under normal conditions because their hepatopancreas can produce L-gulonolactone oxidase. They also suggested that the fish may require dietary ascorbic acid to maintain health under abnormal conditions. However, later it was found that

intraperitoneal injection did not result in an increased concentration of ascorbate in tissue (Dabrowski, 1991) . It is also suggested that the metabolic rates induced by feeding is the primary factor regulating ascorbate requirements in the common carp.

Dabrowski *et al.* (1988) found that the common carp larvae require vitamin C for normal growth unlike juveniles and adults of this species. Reduction in ascorbic acid requirement as the fish grow older has been reported in the rainbow trout, *Oncorhynchus mykiss*, also (Sato *et al.*, 1978; Waagboe *et al.*, 1989). The ontogenic trend of ascorbate has been quantified by Dabrowski (1992) in three fresh water fishes: roach (*Rutilus rutilus*), white fish (*Coregonus lavaretus*) and Arctic charr (*Salvelinus alpinus).* Total ascorbate declined from 150 to 5 µ/g as newly hatched larvae grew to become several months old juveniles and it could not be reversed by increasing the dietary ascorbic acid.

In catla, decrease in concentration of ascorbic acid in the lens of the eye with age is reported by Chowdhary and Sahu (1990). This is related to the function of the organ rather than the requirement.

In crustaceans also similar results have been obtained. A limited ability by the shrimps to synthesise ascorbic acid has been suggested by Lightner *et al.* (1979), which in young shrimp is insufficient to meet the requirements, but is apparently sufficient to meet ascorbic acid requirement in larger shrimp (≥12g). So it is likely that the requirement for ascorbic acid decreases with age. He and Lawrence (1993) who studied vitamin C requirement in two size groups of *Penaeus vannamei* found that ascorbic acid requirement decreases as the size increases. However, in the blue crab (*Callinectes sapidus*) it is reported that the tissue concentration of ascorbic acid does not change with size (Coglianese and Neff 1981)

2.3. Tissue storage of vitamin C

Dabrowski and Kock (1989) determined the sites of absorption of ascorbic acid in the Rainbow trout. They found that 20.7% of the total ascorbic acid was absorbed in the stomach, 23.4% in the pyloric ceacae, 21.9% in the middle intestine and 20.1% in the posterior intestine.

The proportional changes of tissue vitamin C with dietary vitamin C are reported by Dabrowski (1990). The maximum concentration of ascorbate was found in brain followed by liver, skin and muscle, of which brain ascorbic acid content does not change with dietary levels of ascorbic acid , whereas in liver, skin and muscle the level changes (Alexis *et al.*, 1990). In adult rainbow trout the maximum concentration was found in the female gonads followed by the brain, head kidney, testes, spleen, liver, eyes, red muscle and heart tissue.

In crustaceans also change in whole body ascorbic acid content with dietary vitamin C has been reported in *P.monodon* (Shiau & Jan , 1992a) and *P.vannamei* (He & Lawrence,1993). While studying the effect of dietary ascorbic acid supplementation on the accumulation of vitamin C in fish tissue in *Coregonus laveratus*, it is suggested by Dabrowski (1990) that the optimum dietary concentration of vitamin C is equivalent to that allowing the maintenance of steady - state tissue concentration in larval and juvenile fish.

2.4 Deficiency of vitamin C

L-ascorbic acid basically acts as a biological reducing agent for hydrogen transport. It is necessary for formation of collagen and normal cartilage. It is also essential for maturation of erythrocytes (Halver, 1972).

The symptoms resulting from the deficiency of ascorbic acid such as skeltal deformities (scurvy), poor wound healing , etc., should be attributed to impaired collagen formation arising

from lack of ascorbic acid (Sato *et al.*,1982). The deficiency symptoms exhibited by various species of fishes are given in the Table-I.

Early hypovitaminosis C can be detected by examination of fragile support cartilage in the gill filament, before clinically acute symptoms become noticeable. However the best tissues for routine clinical analysis to assess vitamin C status is the anterior kidney.

In the case of shrimps, the deficiency of vitamin C causes black death disease, which was first observed in P.*japonicus* by Deshimaru and Kuroki (1976).

Hunter *et al.* (1979) demonstrated that dietary ascorbic acid was required for protein hydroxylation in collagen formation and that black death is related to collagen underhydroxylation, which culminates in the melanization of haemocyte lesions. The disease invariably resulted in the death of affected animals, once the signs become visually apparent. Baticoides *et al.* (1992) are of the opinion that the blackening of the gills associated with black death may be due to the heavy deposition of the black pigments at the sites of heavy haemocyte activity.

The deficiency symptoms exhibited by various species of prawns are given inTable-II.

2.5 Quantitative dietary requirement of vitamin C

The requirement of vitamin C was first demonstrated in Salmonids by Halver (1989). He suggested that the requirement may be related to growth rate, size of animal, stress and also the presence of certain nutrients in the diet.

2.5.1 Requirement of vitamin C for normal growth and survival

In the case of fishes, growth rate is the most important parameter affected by vitamin C deficiency (Sato *et al.*, 1978; Roselund *et al.*, 1990; Shiau and Jan, 1992).An exception to the

TABLE-I. SCURVY SYMPTOMS EXHIBITED BY DIFFERENT SPECIES OF FISHES.

SPECIES	SCURVY SYMPTOMS	REFERENCE
Cyprinus carpio	Caudal fin erosion and deformed gill arches in larval stage	Satoh (1991)
Ctenopharyngodon idella	Haemorrhages at the base of pectoral and ventral fins	Ding (1991)
Cirrhinus mrigala	Poor growth, high mortality rate, severe haemorrhages, fin necrosis, increased pigmentation, spinal flexures, anorexia, lethargy,unbalanced swimming	Mahajan and Agarwal(1980)
Clarias lazera	Spinal malformation and infection of posterior cranial symphysis. The bones of the head are lighter than those in normal fish and a hollow sound is produced when it is taped against head surface.	Roberts and Bullock (1989)
Ictalurus punctatus	Scoliosis, lordosis, reduced bone collagen, dark skin colour,fin erosion, increased susceptibility to bacterial infections.	Wilson (1991)
Salmo salar	Increased mortality,slow growth, lethargy, scoliosis, lordosis,broken backs, anaemia.	Helland *et al.* (1991)
Anguilla sp	Haemorrhage in fins, head and skin and lower jaw erosion	Arai (1991)
Seriola quinqueradiata	Scoliosis, dark colouration,haemorrhage on the body surface.	Shemino (1991)

{cont...}

Lates calcarifer	Dark colouration, loss of equilibrium, and halted growth,broken back	Boonyaratpalin *et al.* (1990)
Plecoglossus altivelis	Loss of appetite, mild exophtalmia, eyes and fin base haemorrhage, congestion of the back of the head, gill operculum and lower jaw erosion.	Kanzawa (1991)
Sciaenops ocellatus	Retinal and lenticular lesions,lack of normal eye globe rigidity,intra-ocular haemorrhages, etc.	Collins *et al.* (1993)
Stizostedion vitreum	Retarded growth, increased mortalities, skeletal deformities,lordosis,twisted cartilage of gill filaments, extreme discolouration of vertebrae.	Mac connell *et al.* (1993)
Cichlosoma urophthalmus	Dark colouration, short opercle, haemorrhages in eyes, head and fins, loss of scales,exophthalmia, swollen abdomen, scoliosis, change in head bones.	Chaveze-de-Martinez (1990)
Scophthalmus maximus	Deposition of tyrosine crystals in tissue and high mortality.	Coustans *et al.* (1990)
Oreochromis niloticus	Erratic, unbalanced and convulsive movements, haemorrhage around mouth, eyes, fins and caudal fin together with general lethargy and caudal fin erosion.	Soliman *et al.* (1994)

above statement is *Oreochromis mossambicus* where survival but not growth was affected by ascorbic acid deficiency (Oyetoyo, 1985).

Deshimaru and Kuroki (1976) who made the first step towards the defining specific vitamin requirement of prawns, found in *P.japonicus* that the highest growth was obtained for a diet that contained no ascorbic acid and survival was the most important parameter affected by ascorbic acid deficiency. Similar results were obtained in *M. rosenbergii* by Heinen (1988) and in *P. vannamei* by He and Lawrence (1993).

The requirement of ascorbic acid by the larvae of *M. rosenbergii* is reported by New (1995). He had suggested a method of enriching *Artemia* nauplii with ascorbyl palmitate. Although there was no significant differences in growth and survival of larvae, a positive effect was noticed in the physiological condition of the post-larvae which indicated vitamin C requirement during metamorphosis. According to him a high supplementation of vitamin C might enhance production characteristic under stress situations.

The requirement of vitamin C vary depending upon the environment. It was found that the ascorbic acid content was high in brackish water shrimp *Metapenaeus sp.* compared to fresh water prawn, *Macrobrachium* (Dey *et al.*, 1988).

Opinions differ among scientists regarding the requirement of vitamins as such for commercial culture of prawns. For cultivating *M.rosenbergii*, it is reported that there is no need for vitamin premix in the diet, where the rearing is carried out in earthen ponds or in concrete ponds (Boonyaratpalin and New, 1980). Trino *et al.* (1992) reported that in a modified extensive culture system, it would be more profitable to use diet without vitamin supplementation for *P. monodon*. This may be because of the ability of certain microalgae for heterotrophic production of ascorbic acid (Running *et al.*, 1994) and its utilisation by the prawn as an ascorbic acid source (Magarelli *et al.*, 1978; Baticoides *et al.*, 1992).

Table-II. THE DEFICIENCY SYMPTOMS EXHIBITED BY VARIOUS SPECIES OF PRAWNS

SPECIES	SCURVY SYMPTOMS	REFERENCE
Peneaus japonicus	Development of greyish-white colour on the margin of the carapace, the lower part of the abdomen and tip of the walking legs followed by mortality.	Deshimaru and Kuroki (1976)
P. californiensis and *P. stylirostris*	Reduced growth rate, poor FCR, decrease resistance to stress and reduced capability to heal wound	Lightner *et al.* (1979) Magarelli *et al.* (1979)
P. indicus	Reduced feed intake, poor FCR, high incidence of post moult deaths, atrophy of muscle, hepatopancreas, blackening of gills	Gopal (1986)
P. monodon	Reddish brown to black discoloration and atrophy of gills. Dorsal side of the body may be covered with a fog like substance.	Baticoides *et al.* (1992)
P. vannamei	Incomplete moulting, abnormal coloration, swollen hepatopancreas, The shrimp appears motionless and are unresponsive to disturbances	He and Lawrence (1993)
P. chinensis	White-black spot disease	Gao *et al.* (1992)
Macrobrachium rosenbergii	Higher incidence of small cuticular black or dark brown lesions, moulting of only the abdomen (or only the posterior part of it) for prawns dying while trying to moult and presence of subcuticular blotches in the rostrum and other part of non-moulting animals, these blotches usually being white in living animals and brown in dead ones.	Heinan (1988)

Requirement of vitamin C by various species of fishes and shrimps are given in Table.III .

2.5.2 Requirement of vitamin C for disease resistance

Vitamin C plays an important role in disease resistance, provided the fish are fed with vitamin C at a level much higher than that required for normal growth in *Oncorhynchus mykiss*(Navarre and Halver, 1989 ; Anggawati *et al.*, 1990), *Salmo salar* (Erdal *et al.*, 1991 ; Verlhac and Gabauddin, 1994),*Ictalurus punctatus* (Durve and Lovell, 1982; Li and Lovell, 1985) and *Cichlosoma urophthalmus* (Chavez-de-Martinez, 1990).

However, in the case of *I.punctatus* there are difference of opinion. Durve and Lovell(1982) who studied vitamin C requirement for disease resistance in channel cat fish, when infected with *Edwardsiella tarda* at two temperature regimes found that whereas only 30 mg vitamin C / kg diet was enough for normal growth, 150 mg/kg diet was required to increase disease resistance. They also found that vitamin C requirement for resistance to infection was possibly higher when the fish were infected at lower temperatures, where the natural resistance was less than when infected at a temperature near optimum for the natural resistance of the fish. Li and Lovell (1985) also got similar results with *E.ictaluri*, but the disease resistance was enhanced only at a level of 3000mg dietary vitamin C/kg. But Johnson and Ainsworth (1991) demonstrated that an elevated dietary level of ascorbic acid could not influence disease resistance against *E.ictaluri.* This contradictory results led Li *et al.*, (1993) to further evaluate the results and they found that elevated dietary vitamin C concentrations did not improve resistance of channel cat fish against *E.ictaluri* infection.

The effect of vitamin C on stress induced immunological responses was studied in species such as *Salmo salar* (Sandnes and Waagboe, 1991; Thompson *et al.*, 1993) and *Cyprinus carpio* (Dabrowski *et al.*, 1991) and found that dietary vitamin C could not ameliorate stress induced immuno-suppression.

Table-III. THE REQUIREMENT OF VITAMIN C BY VARIOUS SPECIES OF FISHES AND SHRIMPS

SPECIES	TYPE OF VITAMIN C	REQUIREMENT (mg AAE/kg dry diet)	REFERENCES
Cirrhinus mrigala	L-ascorbic acid	650-750 mg	Mahajan and Agrawal (1980)
Oncorhynchus mykiss	L-ascorbic acid	100 mg	Halver (1972)
O. mykiss	L-ascorbyl-2-So_4	210 mg	Sato *et al.* (1991)
Salmo salar	L- ascorbic acid	50 mg	Lall *et al.* (1990)
S. salar	Ca- ascobate-2-monophosphate	10-20 mg	Sandnes *et al.* (1992)
Ictalurus punctatus	L-ascorbic acid	60 mg	Halver (1989)
Tilapia aurea	L-ascorbic acid	50 mg	Stickney *et al.* (1984)
Oreochromis mossambicus	L-ascorbic acid	400-500 mg	Oyetoyo (1985)
O. aureus	L-ascorbic acid	79 mg	Shiau and Jan (1992)
O. niloticus	L-ascorbic acid	1250 mg	Soliman *et al.* (1994)
Cichlosoma urophthalmus	L-ascorbic acid	40 mg	Chavez-de-Martinez (1990)
Stizostedion vitreum	L-ascorbic acid	96 mg	Mac Connell *et al.* (1993)
Penaeus japonicus	L-ascorbic acid	3000 mg	Deshimaru and Kuroki (1976)
P. japonicus	Mg-L-Ascorbyl-2-Phosphate	215-430 mg	Shigueno and Itoh (1988)
P. californiensis	L-ascorbic acid	5000 mg	Guary *et al.* (1976)
P. stylirostris	L-ascorbic acid	1000 mg	Lightner *et al.* (1979)
P.indicus	L-ascorbic acid	400 mg	Gopal (1986)

{cont..}

P. monodon	L-ascorbic acid	2000 mg	Shiau and Jan (1992)
	L-ascorbyl-2-Po_4-Mg	100 - 200 mg	Catacutan and Pitogo (1994)
P. vannamei	L- ascorbyle-2-Polyphosphate	120 mg	He and Lawrence (1993)
Macrobrachium rosenbergii	L-ascorbyl-2-monophosphate L-ascorbyl-6-palmitate	104.3mg	D' Abramo *et al.* (1994)

2.5.3. Requirement of vitamin C for reproduction.

Hilton *et al.* (1979) found the highest concentration of ascorbic acid in female gonads among different tissues tested in *Oncorhynchus mykiss* and suggested a critical function of this vitamin in reproduction.

Sandnes and Braekkam (1981) studied the ascorbic acid concentration in samples taken from different stages of the ovaries of the cod (*Gadus morrhua*) and found that during early juvenile stages ascorbic acid concentration decreases and thus, demonstrated the importance of ascorbic acid in oogenesis.

The first report on actual effect of ascorbic acid on reproduction came from Sandnes *et al.* (1984), who studied the effect of ascorbic acid supplementation in broodstock feed on reproduction of *O.mykiss*. Although there was no difference in time of ovulation between supplemented and deficient groups, egg number and egg size were lower in group without ascorbic acid in the feed. They have suggested that salmonid broodstock feeds should be fortified with at least 100mg vitamin C/kg diet so as to give a concentration of 20 microgram vitamin C/g wet weight in mature eggs for normal development. Similar results were obtained by Eskelinen (1989) Blom and Dabrowski (1993), Mangor *et al.* (1994) and Dabrowski and Blom (1994). It is also reported that deficiency of vitamin C in maturing fish could affect lipid metabolism (Waagboe *et al.*, 1989).

The role of vitamin C in reproduction has been demonstrated in shrimps also. Cahu *et al.* (1991) have reported on the role played by ascorbic acid in collagen synthesis during embryogenesis in *P.indicus*. The egg number, ascorbic acid concentration in eggs and hatchebility were higher for brood stock fed with a higher vitamin C diet.

In ablated *P.japonicus*, it was found that the dietary ascorbic acid could not improve gonado-somatic index, but was required to resist the stress caused due to eye-stalk ablation. The ascorbic acid deficient prawns showed higher mortality after ablation (Alava *et al.*, 1993).

2.6 Role of vitamin C as an antitoxic agent.

It has been observed that the tolerance of the fishes to nitrite toxicity is increased as ascorbic acid concentration in the diet increases in *O.mykiss* (Blanco and Meade, 1980) and *I.punctatus* (Wise and Tomasso, 1988). Possibly ascorbic acid acts in the reduction of methenoglobin to haemoglobin and also it has a protective effect against stress in fishes. For *O.mykiss* the safe level suggested is 200 mg vitamin C/kg diet (Blanco and Maede, 1980). Vitamin C was also found to be antitoxic to chalkones in *Lepidocephalichthys thermalis* (Khan and Ali, 1980).

2.7 Different forms of Vitamin C used in aquaculture feeds.

The ascorbic acid has a structure which is extremely labile. So environmental influences such as time, temperature, oxygen, pH, light, presence of trace elements etc. can easily oxidise it. Therefore, large scale destruction of ascorbic acid occurs during feed processing and storage (Hilton *et al.*, 1977) causing much economic loss. This necessitated the use of chemically stable forms such as phosphate or sulphate derivatives of ascorbic acid in aquaculture feeds.

Phosphate derivatives of vitamin C have been tested in many species of fishes and have been found effective in *S.salar* (Waagboe *et al.*, 1991), in *O.mykiss* (Sandnes and Waagboe, 1991; Sato *et al.*, 1991; Miyasaki *et al.*, 1993; Volker and Fenster, 1994), in *I.punctatus* (Lovell and Ell Naggar, 1990; Ell Naggar and Lovell, 1991; Wilson *et al.*, 1989; Buddington *et al.*, 1993), and in *S.quinqueradiata* (Kanazawa *et al.*, 1992).

These derivatives were found to be 83 times stable than free ascorbic acid at 25 °C and 43 times at 40 °C in aquaculture feeds (Grant *et al.*, 1989). L-ascorbyl-2-phosphate has been granted government clearance (FDA) for use in fish feeds in U.S.A, (Lovell, 1989).

Sulphate derivatives of ascorbic acid is another form tested for use in aquaculture feeds.Although its stability is almost equal to phosphate derivatives (Shiau and Hsu, 1993), in many species of fishes its utilisation is poorer compared to phosphate derivatives (Lovell and El

Naggar, 1990; Waagboe *et al.*, 1991; El Naggar and Lovell, 1991) and also L-ascorbic acid (Dabrowski *et al.*, 1990). This may be because of the fact that these derivatives are absorbed only after hydrolysis to ascorbic acid in intestine, and phosphate derivatives are more easily hydrolysed in the intestine of fishes than sulphate, because of the lack of sulphate activity in the gastro-intestinal tract of the fishes (Dabrowski and Kock, 1989; Dabrowski *et al.*, 1990; Sandnes and Waagboe, 1991).

In *P.monodon*, L-ascorbyl-2-polyphosphate is effectively utilised as ascorbic acid source (Reddy, 1992; Catacutan and Pitogo, 1993). The sulphate derivatives are only 25% effective as phosphate derivatives in *P.monodon* (Shiau and Hsu, 1993). Phosphate derivatives were utilised effectively in *P.vannamei* (He and Lawrence, 1993) and in *M.rosenbergii* (D ' Abramo *et al.*, 1994) also.

Ascorbyl palmitate was found to be effectively utilised by *O.mykiss* (Albreketsen *et al.*, 1988) and *M.rosenbergii* (D ' Abramo *et al.*, 1994).

Another form of vitamin C used in aquaculture feed is the coated form of vitamin C. For e.g. ethyl cellulose coated ascorbic acid (Wilson *et al.*, 1989; Waagboe *et al.*, 1991), polymer coated ascorbic acid (Skelback *et al.*, 1990), glyceride coated ascorbic acid (Soliman *et al.*, 1986) and hydrogenated vegetable oil coated ascorbic acid (Kanazawa, 1992). Although the stability of these physically stabilised forms are much lower compared to chemically stabilised forms such as phosphates and sulphates (Wilson *et al.*, 1989; Waagboe *et al.*, 1991) with respect to available ascorbic acid per unit weight of the compound and cost performance, these are superior to the phosphate and sulphate derivatives (Igarashi, 1994).

3. MATERIAL AND METHODS.

A study was conducted to find out the optimum requirement of vitamin C for *Macrobrachium rosenbergii* juveniles and to characterise the deficiency syndromes, if any, in them. For the purpose of the experiment, a stable form of vitamin C namely CVC F-90 produced by M/S Takeda Vitamin and Food Asia PTE Ltd, Japan was used. The experiment was conducted for a period of 56 days at the Freshwater Prawn Hatchery of the College of Fisheries, Kochi.

3.1 Experimental animals.

The experiment was conducted using *M.rosenbergii* juveniles of a single batch obtained from the hatchery. The larvae obtained from a berried female collected from the wild were reared in the hatchery in fibreglass tanks provided with clear water re-circulation through a biological filter, at a salinity of 12-14 ppt. The larvae were fed with thelly *(Metapenaeus dobsonii)* meat-hen's egg suspension at an interval of 3 hours during day time and with Artemia nauplii during night. The rearing of the larvae was done according to the method reported by Nair and Thampi (1988) and Sebastian (1990).

After settling, the post larvae were reared in 1.2 tonne, flat bottomed, oval fibreglass tanks at a stocking density of 500/sq.m. They were fed with pelleted feed with clam meat as the protein source twice daily at a rate of 20% of body weight. Every morning before feeding, the bottom of the tanks were cleaned and 75% water was exchanged with clear water. Moderate aeration was given using diffuser stones. Some dried twigs were provided at the bottom of the tank to act as substratum and for providing shelter for newly moulted individuals so as to reduce cannibalism (Smith and Sandifer, 1975).

3.2 Experimental rearing facilities.

The experiment was conducted in the hatchery shed with provisions for subdued light penetration and continuous air and water supply. The floor was having a gentle slope towards the sides for easy drainage.

Circular, flat bottomed, fibreglass tanks with the following specifications were used for the experiment.

Capacity : 83 lit

Diameter : 55 cm

Height : 35 cm

Thickness of wall : 1mm

Rimwidth : 3cm

Colour : Aquamarine.

Fresh water pumped from a well was used for the experiment. The water was first pumped into a sump for settling of suspended impurities and then pumped to an overhead tank to facilitate water supply by gravity. Before filling, the water was filtered using fine meshed nylon bolting silk to prevent algae or any debris entering the tanks. The tanks were filled up to a height of 20 cm and the level was maintained throughout the experiment.

Flat square tiles of 20×20 cm size were placed in an inclined position inside the tanks using stones to provide substratum and shelter for the post larval prawns as shown in Plate-I

Aeration was provided in the tanks using diffuser stones and plastic tubes attached to air distribution system of the hatchery . Care was taken to provide uniform aeration in all the tanks using control valves. The tanks were arranged in the shed as shown in Plate-II.

3.3 Experimental diets

3.3.1 Diet formulation

The diets were formulated as reported by Sherief *et al.* (1992) in which casein and egg albumin were the protein source. Eight semi-purified and isoproteinacious diets were prepared incorporating eight levels of vitamin C, in the form of CVC F-90, from 0 - 500 mg/kg diet. The carrageenan used as binder in the basal diet was replaced with carboxy methyl cellulose and the lipid source, sun flower oil, was replaced with corn oil for the experiment.The composition of the experimental diet is given in Table-IV.

PLATE I

EXPERIMENTAL TANK USED FOR THE STUDY

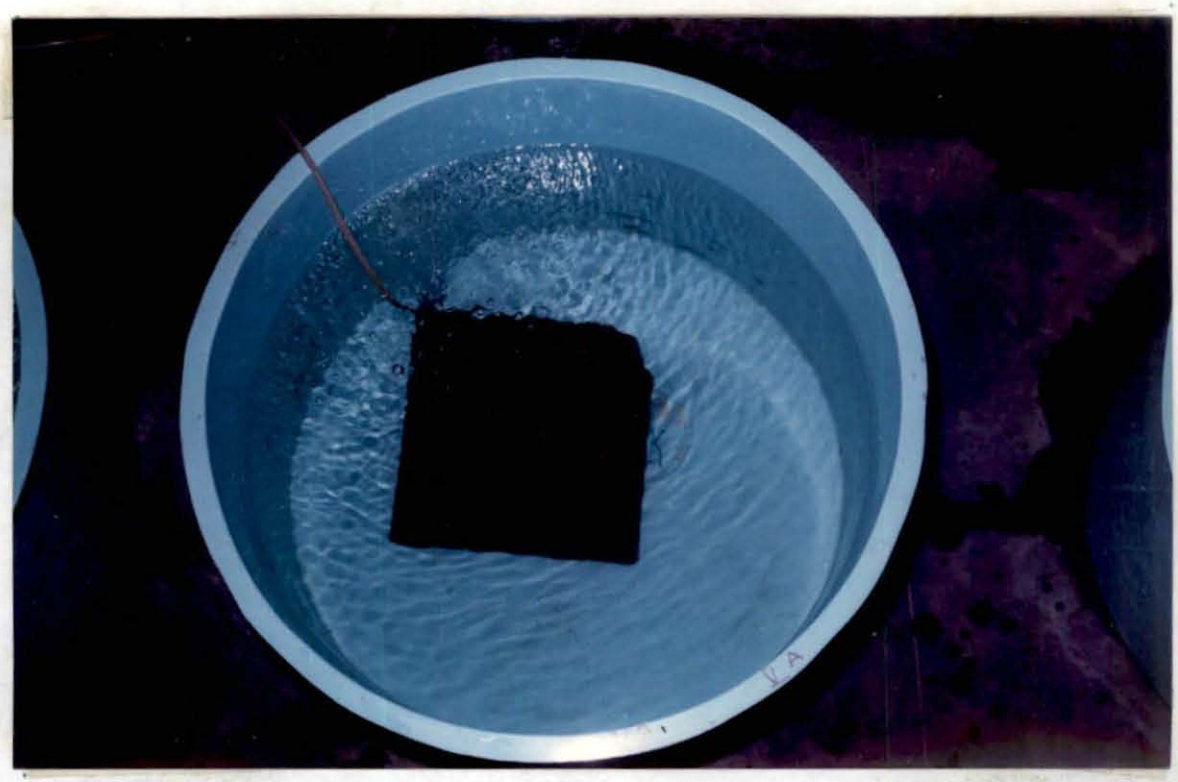


PLATE II

ARRANGEMENT OF EXPERIMENTAL TANKS

Casein when combined with egg albumin was found to be a complete protein source and was easily assimilated by *M.rosenbergii* (Hilton *et al.*, 1984; Briggs *et al.*, 1988). For the experiment vitamin free casein was used to avoid errors due to vitamins present in casein (He and Lawrence, 1993; D' Abramo *et al.*, 1994).

In order to satisfy the requirement for various polyunsaturated fatty acids, a mixture of corn oil and cod liver oil was used as the lipid source. The polyunsaturated fatty acids belonging to ω_3 and ω_6 fatty acids were found to be highly essential for the growth of *M.rosenbergii* (Reigh and Stickney, 1989; D' Abramo and Sheen, 1993). In addition to this, cholesterol was also incorporated in the diets at a rate of 0.5%, since the studies by Briggs *et al.* (1988) and Sherief *et al.* (1992) showed that cholesterol is required in the diet of *M.rosenbergii.*

The carbohydrate source selected was α corn starch, since polysaccharides were found to be more easily assimilated than monosaccharides by prawn (Pascal *et al.*, 1983; Alava and Pascal, 1987).

Hydrogenated vegetable oil coated ascorbic acid with 10% coating produced by M/s Takeda Vitamin and Food Asia PTE LTD, Japan was used for the experiment. Since it was found to be highly stable during processing and storage of shrimp feeds (Khajarern and Khajarern, 1994). With respect to cost also, this ascorbic acid source was found to be better compared to ascorbic acid derivatives such as polyphosphates (Igarashi, 1994). In the experimental diet the basal vitamin -mix composition except vitamin C was the same as reported by Kanazawa *et al.* (1982). (Table-V). To this mix CVC F-90 was added at levels 0,50,100,125,150,200 and 500mg/kg diet and α cellulose was used as the filler.

Mineral-mix is the same as used by Stahl and Aheran (1978) and its composition is given in Table-VI.

Table-IV. COMPOSITION OF EXPERIMENTAL DIET

	DIETS							
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
α corn 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
Cholestrol	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	4.0	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
α 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

Table-V. COMPOSITION OF VITAMIN MIX

VITAMINS	QUANTITY (mg)
Thiamine-Hcl	5.0
Riboflavin	8.0
Para amino benzoic acid	10.0
Biotin	0.4
Inositol	400
Niacin	40
Calcium pantothenate	60
Pyridoxine-Hcl	12
Menadione	4
β-Carotene	9.6
Calciferol	100
Vitamin B_{12}	0.1
Folic acid	0.9
Choline chloride	600
α- Tocopherol	20
Total	1270

Table-VI . COMPOSITION OF MINERAL MIX

MINERALS	QUANTITY(mg)
Calcium biphosphate	40
Calcium lactate	1489.6
Ferric citrate	118.8
Magnisium sulphate	528
Potassium hydrogen phosphate	1016
Sodium chloride	745.6
Sodium biphosphate	41.6
Aluminium chloride	0.4
Potassium chloride	0.4
Cuprous chloride	0.4
Manganous sulphate	3.2
Cobalt chloride	4.0
Zinc sulphate	12.0
Total	4000

3.3.2 Processing and storage of diets.

While processing and storing utmost care was taken to minimise the loss of vitamin C. All the ingredients were first powdered and sieved through 250 micron sieve separately. Then they were weighed accurately in an electronic balance according to percentage composition. Sufficient boiling water was added to ∝-corn starch first so as to dextrinise it. Then all the ingredients except vitamin mix, carragenan, CVC F-90 and oils were added to it and made into a dough. CVC F-90 was thoroughly mixed with caragenan and added to the dough when the temperature was almost 50-60°C together with oil and vitamin basal mix. This was done to prevent temperature degradation of vitamins. Now all the ingredients were thoroughly kneaded until a consistency suitable for pelletising was obtained. The dough was then extruded through a hand pelletizer with 3mm die into an enamel tray and dried at 50°C for 12 hrs. to a moisture content of <10 %. After drying, the pellets were broken into pieces and sieved so as to get a particle size of 1-2 mm.

The feed thus obtained was stored in plastic containers covered with black paper in a refrigeration at 4°C to prevent the storage loss of vitamins (Paulraj, 1993). Feed with no vitamin premix was also prepared for the conditioning of experimental animals.

3.3.3 Proximate analysis of the diet.

After preparation, proximate analysis was done to quantify the nutrient levels in the feed. The methods used for the analyses are as follows.From each treatment, 3 samples were analysed and mean value was taken.

Moisture content: By drying the sample at 105 °C for 12 hrs.

Crude protein : By microkjeldhal method.

Crude fat : By solvent extraction method using petroleum ether (BP 40-60 °C) in a soxhelet-extraction apparatus for 6 hrs.

Ash : By combustion at 450 °C for 12 hrs.

Crude fibre : By method of Pearson (1976)

Carbohydrate : By difference in dry weight (Hastings, 1976).

3.4 Experimental procedure.

Healthy and well pigmented juveniles of average weight 61.24 ± 7.43 mg were used for the experiment. The experiment was planned in completely randomised design. 24 tanks were allotted randomly for 8 treatments with 3 replications each. Tanks were filled upto 20 cm height with filtered fresh water and 10 animals were stocked in each tank.

Prior to initiation of the feeding trial, the animals were fed 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 acclimatise the prawns to the experimental semipurified diets (He and Lawrence, 1993). Feeding was done *ad libitum* twice daily during this period.

After conditioning, the prawns were starved for 24 hrs. before weighing, to empty the gut contents which may lead to erroneous values. Weighing was done using standardised blotting technique in an electronic balance [SHIMADZU - LABROR - AEU' - 130V] with an accuracy of 0.0001g.

Feeding was done twice daily in the morning and evening at a rate of 20 % of the body weight during the first 4 weeks, which was reduced to 10 % in the last four weeks. The feed was provided in a petridish. The feed required for a period of one week was weighed out and stocked separately in black containers. Every day before giving feed, the remnants of diet of the previous day before were collected and dried at 60 °C for estimation of actual feed consumed.

The sides and bottom of the tanks were scrubbed to prevent algal growth, and excreta, exuvia and remnants of diet were removed every day before first feeding. About 75 % of water was changed daily.

Moulting, abnormal syndromes, if any, and mortality were noted daily and recorded in the respective charts maintained for the purpose. The weights of dead specimens were also noted for computation of total weight gain.

Growth assessment was done every fortnight using the same procedure as described earlier. During such occasions the tanks, tiles, stones and air-diffuser stones were cleaned and dried, so as to avoid algal growth.

After a period of 56 days, the prawns were captured for final assessment. Prawns under each treatment were pooled and used for estimation of whole body ascorbic acid.

3.5 Water quality analysis

The physico-chemical parameters of water such as temperature, pH and dissolved oxygen were measured daily and total alkalinity, total hardness, total ammonia-nitrogen and total nitrite-nitrogen were measured weekly.

The following methods were used for the water quality analyses:

Temperature	: Using mercury bulb thermometer with accuracy of 0.1° C
pH	: Using universal pH indicator solution.
Dissolved oxygen	: By standard Winkler method (Strickland and Parson, 1972).
Total alkalinity	: By chemical method (Boyed and Pillai, 1984).
Total hardness	: By chemical method (Boyed and Pillai, 1984)
Ammonia-nitrogen	: By photometric method (Boyed and Pillai, 1984).
Nitrite-nitrogen	: By photometric method (Boyed and Pillai, 1984).

3.6 Whole body ascorbic acid analysis.

After the final assessment, the prawns of each treatment were pooled together and three 1 g samples were taken from each treatment for whole body ascorbic acid analyses. . The method used was that reported by Omaye *et al.*(1979).

3.7 Evaluation indices.

3.7.1. Survival rate (SR)

$$SR = \frac{\text{Final number of prawn}}{\text{Initial number of prawn}} \times 100$$

3.7.2. Percentage weight gain : (PGR)

$$PGR = \frac{\text{Average final weight - Average initial weight}}{\text{Average initial weight}} \times 100$$

3.7.3. Specific growth rate (SGR)

$$SGR = \frac{\ln Wf - \ln Wi}{Tf - Ti} \times 100$$

where,

Wi = Initial average weight of experimental animals at day Ti

Wf = Final average weight of experimental animals at day Tf

ln = Natural logarithm

Specific growth rate is expressed as percentage (assuming exponential growth).

3.7.4. Food conversion ratio (FCR)

$$FCR = \frac{\text{Feed in take on dry matter basis}}{\text{Weight gain on a wet matter basis}}$$

3.7.5. Moulting rate (MR) (Petrilla, 1990)

Moult percentage = $m/n \times 100$

m= number of moults

n= initial number of animals.

$$MR = \frac{\text{Moult percentage}}{\text{Mean life of group}}$$

Mean life of group was calculated by adding the number of days each individual survived in a group and then finding the mean.

3.7.6. Whole body ascorbic acid content

Obtained as per methods of Omaye *et al.* (1979).

3.8. Statistical methods

The experiment was planned using completely randomised design and the data were analysed using Analysis of Variance (ANOVA) technique at 1 % level of significance. Pairwise comparison was done using ' t ' test (Snedecor and Cohran, 1968). In order to satisfy the condition for applying ANOVA Arc Sine transformation was done wherever necessary as follows.

$y = \sin^{-1}(x/100)^{0.5}$ where y is the transformed value and x is the observed value in percentages.

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 a curvilinear fashion ($y = a+bx+cx^2$).

4. RESULTS

Since the vitamin C used in the experiment, CVC F- 90, has 10 % coating with hydrogenated vegetable oil, the Ascorbic Acid Equivalent (AAE) of different dietary levels of CVC F- 90 used are 0, 45, 67.5, 90, 112.5, 135, 180 and 450 mg AAE/kg for diets indicated by C_1, C_2, C_3, C_4, C_5, C_6, C_7, and C_8 respectively.

The influence of various levels of dietary ascorbic acid on *Macrobrachium rosenbergii* juveniles has been determined with reference to survival rate, percentage weight gain, specific growth rate, food conversion ratio, moulting rate and whole body ascorbic acid content. The observations made are given in detail in the following sections.

4.1 Effect of vitamin C on survival rate

The survival rates obtained for the different dietary levels of vitamin C are given in Table VII and is graphically represented in Figure I. In order to meet the theoretical requirement for applying analysis of variance technique the rates were subjected to Arc Sine transformation. The survival rates for different dietary levels of vitamin C showed significant difference at 1% level of significance. Subsequent pair wise comparison leads to the conclusion that dietary levels C_1 (0 mg AAE / kg), C_2 (45 mg AAE / kg) and C_3 (67.5 mg AAE / kg) give significantly lower survival rate when compared to the survival rates obtained with dietary levels C_5 (112.5 mg AAE / kg), C_6 (135 mg AAE /kg), C_7 (180 mg AAE / kg) and C_8 (450 mg AAE / kg). The maximum mean survival rate was obtained for the treatment C_8 with ascorbic acid level of 450 mg AAE/kg diet.

4.2. Effect of vitamin C on percentage weight gain

The percentage weight gain obtained by the experimental animals during the study period for various dietary vitamin C levels are given in Table IX and expressed graphically in Figure 2.

TABLE - VII SURVIVAL RATE OF *M. ROSENBERGII* JUVENILES FED WITH DIFFERENT TEST DIETS.

DIETS	REPLICATION	SURVIVAL RATE (%)	MEAN
C_1	1	40	40
	2	30	
	3	50	
C_2	1	50	56.67
	2	60	
	3	60	
C_3	1	60	63.33
	2	70	
	3	60	
C_4	1	70	80
	2	80	
	3	90	
C_5	1	90	90
	2	80	
	3	100	
C_6	1	80	86.67
	2	90	
	3	90	
C_7	1	80	90
	2	90	
	3	100	
C_8	1	90	93.33
	2	100	
	3	90	

TABLE - VIII ANOVA of survival rate

Source	SS	DF	MSS	F
Diets	4237.5927	7	605.3704	7.83
Error	1237.3432	16	77.3339	
Total	5474.9359	23		

Table value of Fat 1% level = 4.03

CD=15.22

Treatment means:

C_1 = 39.1467

C_2 = 48.8467

C_3 = 52.7767.

C_4 = 63.93

C_6 = 68.8567

C_5 = 75.00

C_7 = 75.00

C_8 = 77.7133

The treatments which are not significantly different are connected with vertical lines.

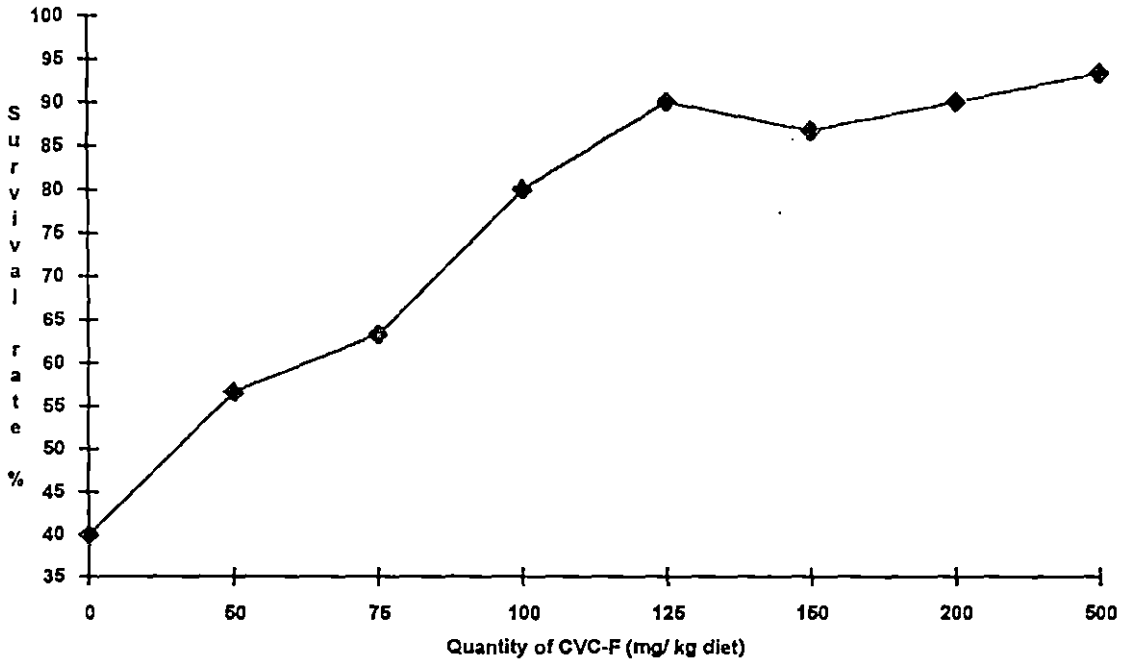


FIG. 1. SURVIVAL RATE OF *MACROBRACHIUM ROSENBERGII* JUVENILES FED WITH DIFFERENT TEST DIETS.

TABLE- IX. PERCENTAGE WEIGHT GAIN OF *MACROBRACHIUM ROSENBERGII* FED WITH DIFFERENT TEST DIETS.

Diet	Replic-ation	Average initial weight (mg)	Average final weight (mg)	Percentage weight gain	Mean
C_1	1	60.77	92.05	51.47	75.34
	2	50.11	88.07	75.75	
	3	60.59	120.46	98.81	
C_2	1	64.12	123.18	92.11	85.92
	2	65.22	126.6	94.11	
	3	70.74	121.35	71.54	
C_3	1	58.78	152.73	159.83	121.15
	2	54.25	132.69	144.59	
	3	79.45	126.35	59.03	
C_4	1	67.59	151.49	124.13	147.54
	2	61.69	161.61	162.06	
	3	59.89	153.49	156.42	
C_5	1	71.89	171.84	139.03	149.07
	2	61.55	176.59	186.91	
	3	69.01	152.70	121.27	
C_6	1	61.38	171.35	179.16	194.92
	2	57.29	159.09	177.69	
	3	47.48	155.49	227.91	
C_7	1	54.13	146.73	171.07	182.34
	2	60.40	169.10	179.97	
	3	60.91	180.29	195.99	
C_8	1	52.39	156.72	199.14	188.25
	2	68.75	174.20	153.38	
	3	59.49	140.76	212.22	

Table- X. ANOVA of percentage weight gain.

Source	SS	DF	MSS	F
Diets	43983.6291	7	6283.3756	7.01
Error	14342.2657	16	896.3916	
Total	58325.8948	23		

Total value of F at 1% level = 4.03

CD= 51.83

Treatment means.

C_1 = 75.34

C_2 = 85.92

C_3 = 121.15

C_4 = 147.54

C_5 = 149.07

C_7 = 182.34

C_8 = 188.25

C_6 = 194.92

Treatments which are not significantly different are connected with vertical lines.

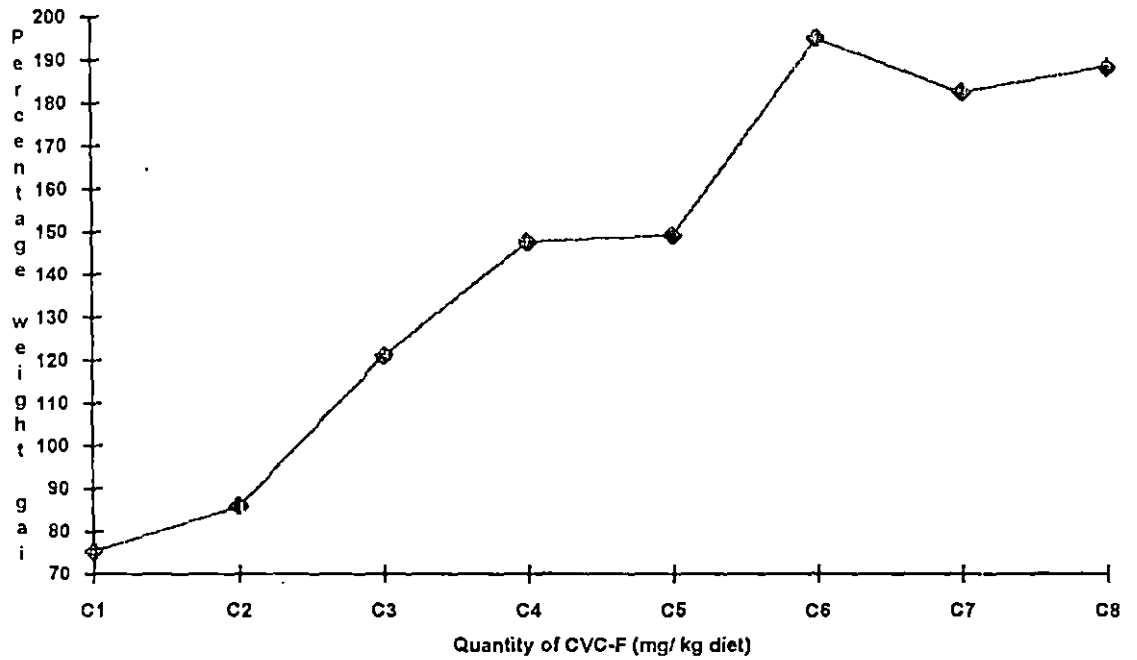


FIG. 2. PERCENTAGE WEIGHT GAIN OF *MACROBRACHIUM ROSENBERGII* JUVENILES FED WITH DIFFERENT TEST DIETS.

TABLE - XI. SPECIFIC GROWTH RATE OF *MACROBRACHIUM ROSENBERGII* FED WITH DIFFERENT TEST DIETS.

Diet	Replicat-ion	Average initial weight (mg)	Average final weight (mg)	SGR	Mean
C_1	1	60.77	92.05	0.7415	0.9919
	2	50.11	88.07	1.0070	
	3	60.59	120.46	1.2271	
C_2	1	64.12	123.18	1.1659	1.1047
	2	65.22	126.6	1.1844	
	3	70.74	121.35	0.9637	
C_3	1	58.78	152.73	1.7051	1.3769
	2	54.25	132.69	1.5972	
	3	79.45	126.35	0.8284	
C_4	1	67.59	151.49	1.4412	1.6143
	2	61.69	161.61	1.7203	
	3	59.89	153.49	1.6815	
C_5	1	71.89	171.84	1.5561	1.6188
	2	61.55	176.59	1.8821	
	3	69.01	152.70	1.4183	
C_6	1	61.38	171.35	1.8833	1.9259
	2	57.29	159.09	1.8238	
	3	47.48	155.69	2.1206	
C_7	1	54.13	146.73	1.7807	1.8523
	2	60.40	169.10	1.8384	
	3	60.91	180.29	1.9378	
C_8	1	52.39	156.72	1.9567	1.8833
	2	68.75	174.20	1.6602	
	3	51.49	160.76	2.0331	

Table -XII ANOVA of specific growth rate

Source	SS	DF	MSS	F
Diets	2.6771	7	0.3824	6.71
Error	0.9116	16	0.057	
Total	3. 5887	23		

Total value of F at 1% level = 4.03

CD=0.43

Treatment means

C_1 = 0.9919

C_2 = 1.1047

C_3 = 1.3769

C_4 = 1.6143

C_5 = 1.6188

C_7 = 1.8523

C_8 = 1.8833

C_6 = 1.9259

Treatments which are not significantly different are connected with vertical lines.

Application of analysis of variance technique on the data (Table-X) showed that the influence of various dietary levels of vitamin C on percentage weight gain was significantly different. The pairwise comparison helped to classify the diets into two major groups. Diets C_1 and C_2 gave a percentage weight gain which is too low that these dietary levels seem to be deficient for *M.rosenbergii*, where as dietary levels C_6, C_7 and C_8 gave comparatively high percentage weight gain. The dietary levels C_3, C_4 and C_5 where falling in-between these two groups. The highest value for percentage weight gain (194.92 %) was obtained for dietary level of 135 mg AAE / kg diet (C_6). Although there is a slight decrease in the value obtained for C_7 (182.34 %), it again increases for dietary level, C_8 (188.25 %).

4.3. Effect of vitamin C on specific growth rate.

Table XI shows the specific growth rate obtained for the various dietary levels of vitamin C. The data are represented graphically in Figure 3. The analysis of variance of the data showed significant difference ($P \leq 0.01$) among the diets with regard to specific growth rate (Table XII). Pairwise comparison showed that treatments C_6, C_7 and C_8 with dietary levels of 135, 180 and 450 mg AAE/kg respectively, were having significant effect on SGR compared to those of treatments C_1, C_2 and C_3 with dietary levels of 0, 45 and 67.5 mg AAE / Kg diet. Treatments C_4 and C_5 gave intermediate values. As in the case of percentage weight gain here also the highest value (1.9259) was obtained for treatment C_6 with 135 mg AAE/kg diet.

4.4 Effect of vitamin C on food conversion ratio.

The food conversion ratio obtained for the various treatments are given in Table- XIII and Figure - 4.

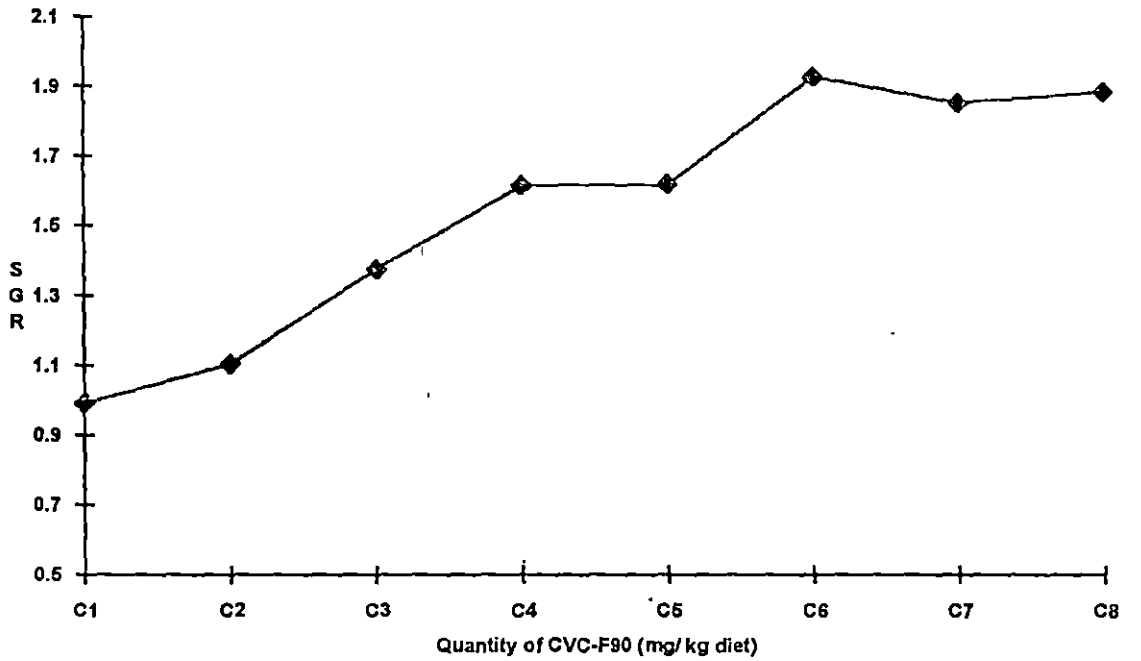


FIG. 3. SPECIFIC GROWTH RATE OF *MACROBRACHIUM ROSENBERGII* JUVENILES FED WITH DIFFERENT TEST DIETS.

TABLE - XIII. FOOD CONVERSION RATIO OF *MACROBRACHIUM ROSENBERGII* JUVENILES FED WITH DIFFERENT TEST DIETS.

Diet	Replica-tion	Total feed consumed (g)	Total weight gain (g)	FCR	Mean
C_1	1	2.1338	0.1448	14.7362	13.3602
	2	2.850	0.1953	14.5929	
	3	3.6512	0.3396	10.7515	
C_2	1	2.9353	0.3837	7.650	8.3098
	2	3.229	0.4041	7.9906	
	3	3.2223	0.3496	9.2888	
C_3	1	4.936	0.7001	7.0504	8.3079
	2	5.2027	0.6581	7.9056	
	3	3.4777	0.3489	9.9676	
C_4	1	5.0201	0.6752	7.435	7.0207
	2	6.133	0.9019	6.8001	
	3	6.0925	0.8924	6.8271	
C_5	1	6.0927	0.9253	6.5846	6.3645
	2	6.2214	1.1206	5.5518	
	3	5.8224	0.8369	6.9571	
C_6	1	5.6758	0.9886	5.7413	5.9783
	2	5.7153	0.935	6.1126	
	3	6.1204	1.0065	6.0809	
C_7	1	5.8001	0.9373	6.1881	5.7477
	2	6.0523	1.0155	5.9599	
	3	6.0827	1.1938	5.0952	
C_8	1	6.0282	0.9882	6.1002	5.895
	2	5.9413	1.0545	5.6342	
	3	6.0994	1.025	5.9506	

Table - XIV. ANOVA of food conversion ratio

Source	SS	DF	MSS	F
Diets	135.031	7	19.29	16.78
Error	18.3932	16	1.1496	
Total	153.4242	23		

Table value of F at 1% level = 4.03

CD = 1.8559

Treatment means :

C_1 = 13.3602

C_2 = 8.3098

C_3 = 8.3079

C_4 = 7.0207

C_5 = 6.3645

C_6 = 5.9783

C_8 = 5.895

C_7 = 5.7477

Treatments which are not significantly different are connected with vertical lines.

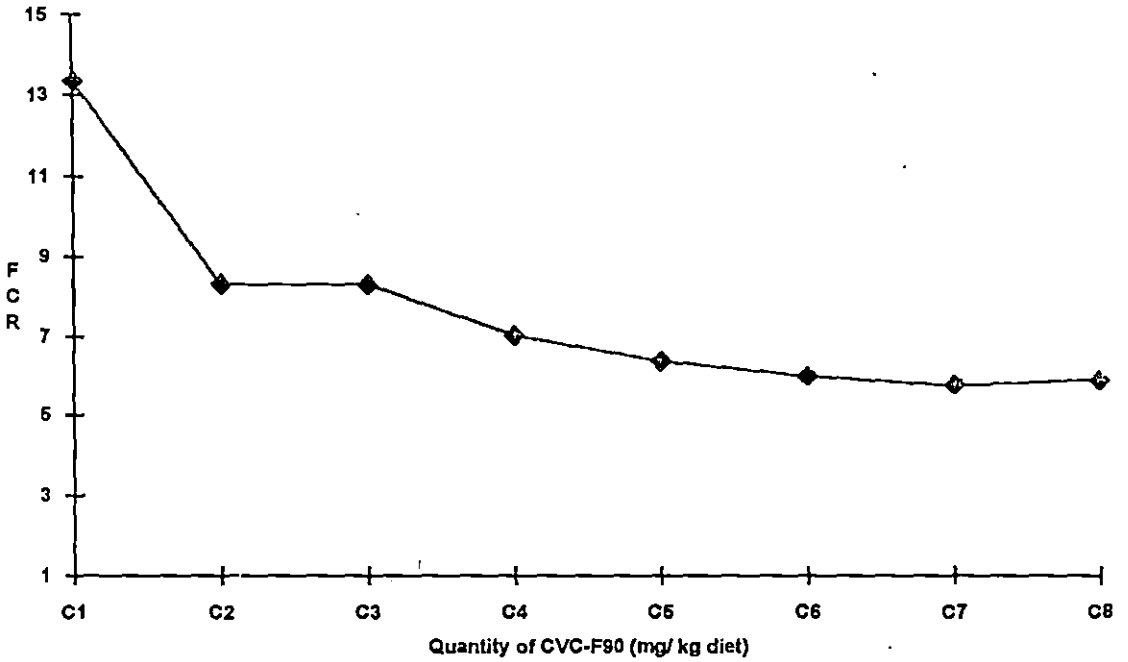


FIG. 4. FOOD CONVERSION RATIO OF *MACROBRACHIUM ROSENBERGII* JUVENILES FED WITH DIFFERENT TEST DIETS.

The analysis of variance of the data obtained showed that the FCR values significantly differed ($P \leq 0.01$) with regard to its influence on FCR (Table-XIV). The pairwise comparison of the mean values showed that the treatment C_1 with 0 level of vitamin C gave a very high value of 13.3602 showing very poor efficiency for food conversion by the experimental animals. The diets C_2 and C_3 also gave significantly higher FCR when compared to other diets. The lowest value for FCR was given by diet C_7 (180 mg AAE/kg diet).

4.5. Effect of vitamin C on moulting rate.

The moult rate of the prawns during the period of study for the various dietary levels of vitamin C are given in Table- XV and is graphically represented in Figure - 5.

The analysis of variance of the moulting rate data showed significant difference among the various dietary levels ($P \leq 0.01$) (Table- XVI). Subsequent pairwise comparison revealed that dietary level of 67.5 mg AAE / kg (C_3) and less than that gave significantly lower moult rate, whereas vitamin C level of 90 - 450 mg / kg diet gave similar values for moulting rate.

4.6. Effect of vitamin C on whole body ascorbic acid content.

The quantity of vitamin C per gram tissue of prawns determined at the end of experiment for the various treatments are presented in Table XVII and Figure 6.

Application of analysis of variance technique in the data showed significant difference ($P \leq 0.01$) between various treatments Table XVIII. Pairwise comparison showed that diets C_1, C_2 and C_3 formed a group which gave significantly lower whole body ascorbic acid content when compared to all the other diets. In general, it can be seen that the whole body ascorbic acid content increases as the dietary levels of vitamin C increases.

TABLE - XV MOULTING RATE OF *MACROBRACHIUM ROSENBERGII* FED WITH DIFFERENT TEST DIETS.

Diets	Replicat-ion	Mean life of group	Moult %	Moulting rate	Mean
C_1	1	32.6	220	6.7485	6.9171
	2	31.0	220	7.0968	
	3	36.2	250	6.9061	
C_2	1	40.5	280	6.9136	6.5622
	2	39.5	250	6.3291	
	3	41.9	270	6.4439	
C_3	1	45.0	330	7.3333	7.2447
	2	48.2	370	7.6763	
	3	46.1	310	6.7245	
C_4	1	46.7	400	8.5653	9.1466
	2	52.6	490	9.3156	
	3	54.4	520	9.5588	
C_5	1	52.9	470	8.8847	9.4115
	2	54.6	530	9.7070	
	3	56.0	540	9.6429	
C_6	1	51.0	500	9.8039	9.7832
	2	51.7	520	10.0580	
	3	52.7	500	9.4877	
C_7	1	54.4	510	9.3750	9.7821
	2	53.1	520	9.7928	
	3	66.0	570	10.1786	
C_8	1	54.0	480	8.8889	9.4006
	2	56.0	530	9.4643	
	3	52.8	520	9.8485	

Table - XVI, ANOVA of moulting rate.

Source	SS	DF	MSS	F
Diets	39.5371	7	5.6482	34.42
Error	2.625	16	0.1641	
Total	42.1621	23		

Table value at of F 1% level = 4.03

CD = 0.7011

Treatment means :

C_2 = 6.5622

C_1 = 6.9171

C_3 = 7.2441

C_4 = 9.1466

C_8 = 9.4006

C_5 = 9.4115

C_7 = 9.7821

C_6 = 9.7832

Treatments which are not significantly different are connected with vertical lines.

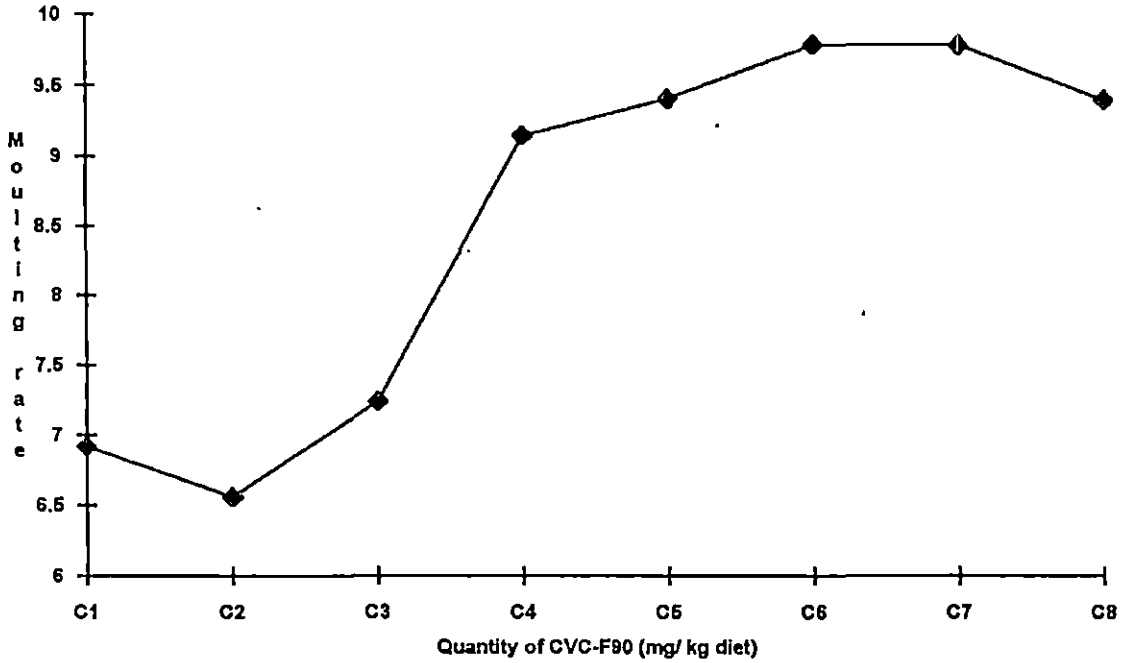


FIG. 5. MOULTING RATE OF *MACROBRACHIUM ROSENBERGII* JUVENILES FED WITH DIFFERENT TEST DIETS.

TABLE - XVII. WHOLE BODY ASCORBIC ACID CONTENT OF *MACROBRACHIUM ROSENBERGII* FED WITH DIFFERENT TEST DIETS.

Diets	Replication	Quantity of ascorbic acid per g tissue(µg)	Mean
C_1	1	1.9677	2.5961
	2	3.8021	
	3	2.0185	
C_2	1	7.6974	5.8121
	2	5.8520	
	3	3.8868	
C_3	1	7.7601	5.8115
	2	3.8886	
	3	5.7857	
C_4	1	9.6465	10.9519
	2	13.4615	
	3	9.7478	
C_5	1	13.7262	13.4218
	2	11.0545	
	3	15.4846	
C_6	1	15.4110	15.7352
	2	13.5841	
	3	18.2104	
C_7	1	14.0681	16.9448
	2	17.519	
	3	19.2472	
C_8	1	15.6855	18.0259
	2	23.1164	
	3	15.2758	

Table - XVIII. ANOVA of whole body ascorbic acid content.

Source	SS	DF	MSS	F
Diets	711. 7281	7	101.6754	16.2577
Error	100.0636	16	6.254	
Total	811.7917	23		

Table value of F at 1% level = 4.03

CD = 4.3288

Treatment means :

C_1 = 2.5961

C_3 = 5.8115

C_2 = 5.8121

C_4 = 10.9519

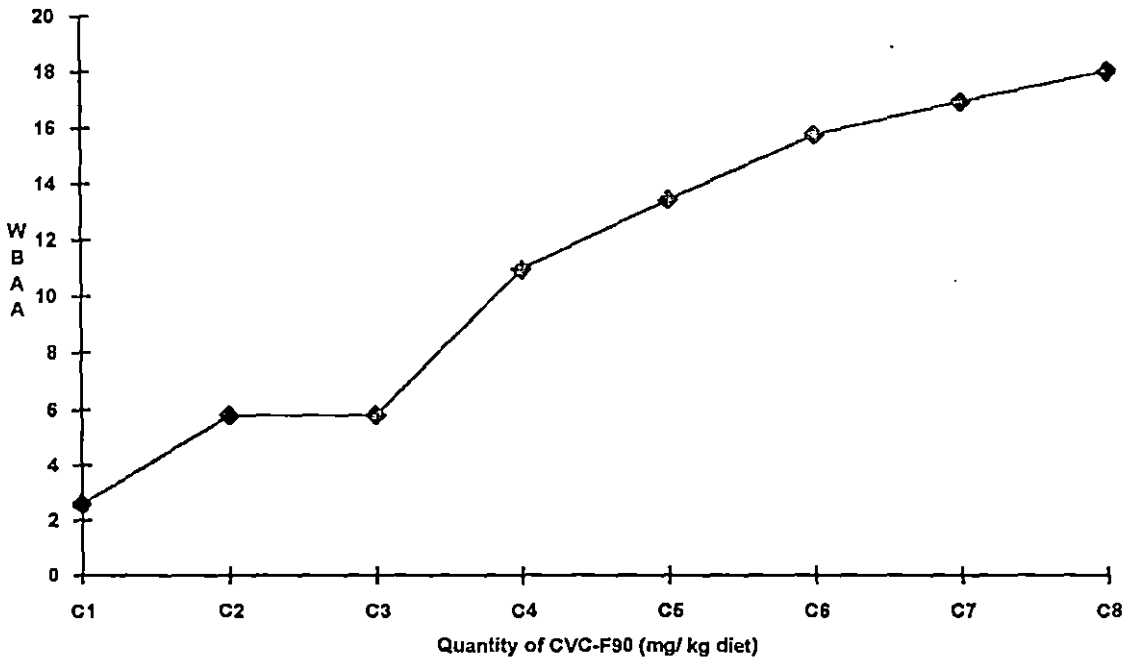
C_5 = 13.4218

C_6 = 15.7352

C_7 = 16.9448

C_8 = 18.0259

Treatments which are not significantly different are connected with vertical lines.



[WBAA- Whole Body Ascorbic Acid.(µg/g tissue)]

FIG. 6. WHOLE BODY ASCORBICACID CONTENT OF *MACROBRACHIUM ROSENBERGII* JUVENILES FED WITH DIFFERENT TEST DIETS.

4.7. Optimum requirement of dietary vitamin C.

The optimum dietary requirement of vitamin C was determined with respect to percentage weight gain, that being the prime factor when commercial culture is considered. The estimation was done by fitting second order polynomial regression line (y= 46.4597 + 1.2607x - 0.0031 x 2). The optimum value obtained is 203.3 mg CVC F - 90 / kg diet or 182.97 mg AAE / kg diet.

4.8. Deficiency symptoms

The prawn fed on diet containing <67.5mg AAE/kg diet showed deficiency symptoms in all these treatments (dietary levels C_1, C_2 and C_3). There were overall reduction in survival rate, percentage weight gain and specific growth rate. The food conversion was poor in these treatments. The moult rate was also lower in these treatments. In the treatments C_1 and C_2 death due to incomplete moulting was observed. In addition, black lesions were observed at the tip of appendages of the prawns. The results showed that a dietary vitamin C level > 90mg AAE/kg diet was required to prevent the development of these symptoms.

4.9. Proximate composition of the diet

The proximate composition of the diet used for the experiment is given in Table -XIX. The average protein content was 47.6±0.27g/100g diet, lipid content 6.73±0.23g/100g diet and carbohydrate content 5.025± 0.6g/100g diet.

4.10. Water quality parameters

The mean value obtained for various water quality parameters such as temperature, pH, dissolved oxygen, total hardness, total alkalinity, ammonia-nitrogen and nitrate-nitrogen are given in Table XX. The water temperature ranged from 26.5^{0}C to 28.3 ^{0}C, pH 7-8, and dissolved oxygen 6.1 - 7.3.

TABLE - XIX. PROXIMATE COMPOSITION OF DIET.

Diet									
Compositon	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	Mean
Protein	48.0	47.2	47.3	47.4	47.6	47.4	47.9	47.7	47.6 ± 0.23
Lipid	7.0	6.7	6.5	7.1	6.9	6.6	6.4	6.6	6.73 ± 0.23
Moisture	9.1	9.0	9.7	8.7	9.0	9.2	8.9	9.1	9.09 ± 0.27
Fibre	5.6	5.7	5.8	5.0	5.0	4.7	4.3	4.1	5.03 ± 0.6
Ash	4.7	5.1	4.6	4.8	4.5	4.9	4.0	3.5	4.51± 0.49
Carbohydrate	25.6	26.3	26.1	27.0	27.0	27.2	28.5	29	26.92± 1.13

TABLE - XX. WATER QUALITY PARAMETERS.

Temperature ^{0}C								
Weeks	1	2	3	4	5	6	7	8
Mean	27.77	27.83	28.04	27.69	27.89	27.74	27.64	28.05
SE	0.37	0.32	0.18	0.36	0.14	0.33	0.51	0.18
Range	27.2 - 28.3	27.5 - 28.2	27.8 - 28.3	27.1- 28.1	27.7- 28.1	27.1- 28.1	26.5- 28	27.9-28.2

Dissolved Oxygen (ppm)								
Weeks	1	2	3	4	5	6	7	8
Mean	6.66	6.84	6.87	6.74	6.74	6.69	6.7	6.9
±SE	0.47	0.31	0.41	0.25	0.30	0.11	0.26	0.22
Range	6.1 - 7.1	6.2-7.2	6.3-7.3	6.3-7.1	6.4-7.2	6.5-6.7	6.5-7.1	6.6-7.3

pH.								
Weeks	1	2	3	4	5	6	7	8
Mean	7.64	7.43	7.64	7.36	7.36	7.57	7.5	7.29
±SE	0.35	0.42	0.35	0.44	0.35	0.32	0.38	0.36
Range	7-8	7-8	7-8	7-8	7-8	7-8	7-8	7-8

Other parameters.

Parameter	Total alkalinity (mg/l as $CaCO_3$)	Total hardness (mg/l as $CaCO_3$)	Ammonia -N (ppm)	Nitrite-N (ppm)
Mean	92.41	111.45	0.026	0.046
± SE	1.8	2.98	0.007	0.012
Range	90.3 - 96.3	106.3 - 116.3	0.02 - 0.04	0.02 - 0.07

5. DISCUSSION

The results obtained from the present experiment conducted to study the influence of dietary vitamin C on survival rate , growth, food conversion ratio, moulting rate and whole body ascorbic acid content of the juvenile *M. rosenbergii* are discussed in the following sections.

5.1 Effect of vitamin C on survival rate

From the result obtained it can be seen that the survival rate of *M. rosenbergii* is significantly influenced by dietary vitamin C. The maximum mean survival rate of 93.33% is obtained with dietary level of 450mg AAE/kg diet. Below a level of 67.5 mg AAE/kg diet, the survival rate is ≤ 70%. It is also observed that most of the mortality occurred in the second fortnight, after which the mortality was less.

The studies conducted in several Penaeid shrimps have revealed the influence of vitamin C on survival. In many cases it has been observed that survival is the prime factor affected by ascorbic acid deficiency, rather than the growth. At a dietary level below 1000mg of vitamin C /kg diet a high mortality was observed in *P. japonicus* (Deshimaru and Kuroki, 1976), *P. californiensis and P. stylirostris* (Lightner *et al.*, 1979).In the case of *P. vannamei*, where L-ascorbyl-2-phosphate was used as vitamin source, a dietary level below 60mg AAE/kg diet gave a very poor survival rate (He and Lawrence, 1993). In the present study it is seen that the survival of the prawns but not the growth was significantly affected by feeding vitamin C deficient diet, and most of the mortality occurred within 21 days after initiation of the experiment. In the earlier studies conducted on *M. rosenbergii* (D'Abramo *et al.*, 1994) it was found that below a level of 50mg AAE/kg diet high mortality occurred when ascorbyl-2-monophosphate calcium salt and ascorbyl-6-palmitate were used as the vitamin source.

The results of the present experiment show that the survival of *M. rosenbergii* is affected greatly when fed on a vitamin deficient diet; and a dietary level of 112.5 mg AAE/ kg diet is required to obtain good survival. The reduced mortality after the second fortnight may be

because of reduced requirement as the prawns grow (Lightner *et al.*, 1979; Magarelli *et al.*, 1979; He and Lawrence,1993).

5.2 Effect of vitamin C on growth

The influence of dietary vitamin C on growth was studied by using two indices, the percentage weight gain and the specific growth rate. The maximum weight gain was obtained with a dietary level of 135 mg AAE/kg diet, above which the growth rate was slightly reduced. The growth rate was significantly lower at a dietary level ≤67.5 mg AAE/kg and moderate with 90 and 112.5 mg/kg diet. Both the indices (percentage weight gain and specific growth rate) changed in the same pattern with dietary level of vitamin C.

Normal growth was observed in some species of shrimps studied, when fed with a vitamin C deficient diet. Deshimaru and Kurokoi (1976) observed maximum growth in *P.japonicus* by feeding a diet that contained no ascorbic acid. In *P.vannamei* also growth was not much affected by feeding vitamin C deficient diet (He and Lawrence, 1993). In *M.rosenbergii,* although the differences were not significant, the growth rate was lower in groups fed with low vitamin C diet and maximum growth was obtained by a level of 100 mg AAE / kg diet (D ' Abramo *et al.*, 1994).But for *P. stylirostris and P. californiensis* the growth was significantly influenced by dietary vitamin C level (Magarelli *et al.*, 1979).

Reduction of growth with high vitamin C is reported for *P. japonicus* (Deshimaru and Kuroki,1976) and *P. indicus* (Gopal,1986). In the present study there was a reduction in growth for a dietary level of 180 mg AAE/kg diet, but it again increased though it didn't reach the maximum with a level of 450 mg AAE/kg diet.

The optimum requirement as determined from percentage weight gain by fitting second order polynomial regression line is 182.97 mg AAE/kg diet. The optimum requirement for the same species obtained with phosphate and palmitate derivatives of ascorbic acid is 104.3 mg AAE/kg diet (D' Abramo *et al.*, 1994). The greater requirement obtained in the present study

may be because of lesser stability of coated form of ascorbic acid, compared to the above derivatives.

5.3 Effect of vitamin C on food conversion ratio.

The feed conversion efficiency by the experimental animals fed on vitaminC deficient diet was very poor. Above a dietary level of 90 AAE/kg diet the food conversion ratios were almost comparable.

A higher FCR for lower level of dietary vitamin C was obtained for *P. indicus* (*Gopal.*, 1986) and for *P. monodon* (Shiau and Jan, 1991). However, in *P.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.

5.4 Effect of vitamin C on moulting rate

The result showed significant influence of dietary vitamin C on moulting rate. Prawn fed ≤67.5 mg AAE/kg diet have much lower number of moults. Most of the mortality were associated with incomplete moulting. Above this level each prawn performed 5-6 moults during the experimental period.

Lightner *et al.* (1979) have reported the inability of ascorbic acid deficient shrimp to hydroxilate sufficient procollagen to produce mature collagen fibres. This may be the reason for poor moulting by such shrimps. The inability of ascorbic acid deficient shrimp to moult completely has been reported in *P. japonicus* (Deshimaru and Kuroki,1976), in *P. indicus* (Gopal ,1986) and in *P. vannamei* (He and Lawrence 1993). In *M. rosenbergii* also this has been reported by Heinan (1988) and D' Abramo *et al.* (1994).

5.5 Effect of vitamin C on whole body ascorbic acid content

In the present study the whole body ascorbic acid level seemed to reflect the dietary level of vitamin C. A tissue level greater than 15µg ascorbic acid /g was required to promote

good growth and survival of experimental animals. It was found to be impossible to deplete the tissue reserves of vitamin C completely even after feeding 0 level vitamin C diet for 56 days.

The studies conducted in *P.californiensis* and *P. stylirostris* of 0.7 g size revealed that tissue levels > 30 µg/g is required for normal growth, while < 20 µg/g will predispose the animal to deficiency diseases. (Lightner *et al.*, 1979). Shiau and Jan (1990) reported that for *P. monodon* of 0.4 g size tissue levels >23 µg/g is required for maximum growth. The studies conducted in *P. vannamie* of two sizes showed that for 0.1g shrimp and 0.5 g shrimp tissue level < 3 µg/g and < 7 µg/g resulted in poor survival respectively. In both cases tissue level greater than 10 µg/g resulted in good growth. Thus it can be seen that the tissue level of ascorbic acid for normal growth vary with the species and also with the size of the shrimp.

From the results of the present study it can be suggested that for *M. rosenbergii* of 0.15g size, a level of >15 µg Ascorbic acid / g tissue is required for maximum growth.

5.6 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 67.5 mg vitamin C / kg diet the growth, survival and food conversion were very poor. Most of the mortality occurred as a result of incomplete moulting in the second fortnight.

The black death disease of ascorbic acid deficient shrimps has been studied by a number of workers(Lightner *et al.*, 1979; Magarelli *et al.*, 1979; Hunter *et al.*, 1979). According to them within four weeks on an ascorbic acid deficient diet, black haemocytic lesions will be observed in loose connective tissues of the body, especially in those areas which are subjected most to mechanical trauma. Once the disease signs are observed, the process is irreversible and the affected animals will develop anorexia and die within 24 to 72 hrs. In the present study black lesions were observed at the tips of the walking legs of the dead specimens. These are areas subjected most to mechanical trauma. These deficiency symptoms were also observed by Heinan(1988) in *M.rosenbergii.*

6. SUMMARY

1.An experiment was conducted to study the influence of dietary vitamin C on growth, survival, food conversion ratio, moulting rate and whole body ascorbic acid content of *Macrobrachium rosenbergii* juveniles and to characterise the deficiency symptoms if any, exhibited by the species.

2. For the experiment, a hydrogenated vegetable oil coated form of vitamin C known as CVC-F.90 with 90% available ascorbic acid was used, since it was cheaper in terms of available ascorbic acid compared to the various derivatives of ascorbic acid.

3. Eight semi-purified diets based on casein and egg albumin were prepared with different levels of vitamin C, i.e., 0, 45, 67.5, 90, 112.5, 135, 180 and 450 mg AAE / kg dry diet, and fed to the experimental animals in three replicates for 56 days.

4. During the course of the experiment moulting and mortality were observed every day and growth was evaluated every fortnight.

5. The growth of *M.rosenbergii* was found to be significantly influenced by different dietary levels of vitamin C. Maximum growth was obtained with a dietary level of 135 mg AAE / kg dry diet.

6. At a dietary level ≤ 67.5 mg AAE / kg dry diet, the survival rate was significantly low. Mortality occurred presumably because of inability of the prawns to moult.

7. Dietary levels ≤ 67.5 mg AAE/ kg dry diet gave very high FCR > 8 which showed poor food conversion efficiency by the ascorbic acid deficient prawns.

8. The moulting rate was poor (2-3 times) for prawns fed ascorbic acid deficient diet. At dietary level >90 mg AAE / kg dry diet, each prawn moulted 5 -6 times during the experimental period.

9. The whole body ascorbic acid level increased as the dietary vitamin C increased. A level of 15µg ascorbic acid / g tissue was found to be required for maximising growth and survival.

10. Below a level of 75 mg CVC - F 90 per kg of dry diet, deficiency symptoms like poor growth, survival and feed conversion, incomplete moulting, and development of black lesions at the tip of walking legs were noticed.

11. Non-linear regression analysis showed the optimum requirement of vitamin C to be 187.92 mg AAE / kg dry diet.

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VITAMIN C REQUIREMENT AND ITS DEFICIENCY SYNDROMES IN *MACROBRACHIUM ROSENBERGII* JUVENILES

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ABSTRACT OF THE THESIS

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8. ABSTRACT

An experiment was conducted to study the influence of dietary vitamin C on growth, survival, food conversion ratio, moulting rate and whole body ascorbic acid content of juvenile *Macrobrachium rosenbergii*, to determine the optimum dietary requirement of vitamin C and to characterise the deficiency syndromes, if any. Juvenile prawns kept under defined environmental conditions were fed casein based semipurified diets containing eight levels of vitamin C. CVC - F 90, a hydrogenated vegetable oil coated form of vitamin C with 90% available ascorbic acid was used as the vitamin source.

Growth, survival, food conversion ratio, moulting rate and whole body ascorbic acid content were found to be significantly affected by dietary vitamin C ($P \leq 0.01$). Below a level of 75 mg CVC - F 90 per kg of dry diet, deficiency syndromes like poor growth, survival and food conversion, incomplete moulting and black lesions at the tip of walking legs were noticed. The maximum weight gain of 194.92% was obtained with a dietary level of 150 mg CVC - F 90 per kg dry diet. Non-linear regression estimate based on percentage weight gain showed the optimum requirement to be 200.3 mg CVC - F 90 per kg dry diet which is equivalent to 182.97 mg AAE / kg dry diet. The tissue ascorbic acid level seemed to reflect dietary levels of VitaminC. About 15 μ gm tissue ascorbic acid / g was required for maximum growth and survival.