Biological Forum – An International Journal **15(4): 926-935(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Comparison of Chitosan Stabilised Ascorbic Acid with Ascorbyl-2-polyphosphate and Crystalline L-ascorbic Acid on Growth, Survival and Whole-body Ascorbic Acid Concentration in the Freshwater Prawn, *Macrobrachium rosenbergii* (de Man)

Hari B.[1]* and Jisha S.[2]
[1, 2]Associate Professor, P. G. & Research Department of Zoology,
Sree Narayana College (Affiliated to University of Kerala), Kollam (Kerala) India.

(Corresponding Author: Hari B.)*
(Received: 28 February 2023; Revised: 17 March 2023; Accepted: 26 March 2023; Published: 15 April 2023)
(Published by Research Trend)

ABSTRACT: The effects of diet supplementation using chitosan stabilized ascorbic acid (CSAA), ascorbyl-2-polyphosphate (ASPP), crystalline L-ascorbic acid (AA) at 250 mg ascorbic acid equivalent (AAE) kg-1 diet and a diet devoid of ascorbic acid (C) was studied using juvenile *Macrobrachium rosenbergii* prawns in a 50-day feeding trial. Ascorbic acid retention (%) after feed processing, leaching, and storage revealed that both CSAA and ASPP were significantly more heat and water stable than AA. Prawns had an initial weight of 64 ±0.02 mg. Prawns fed CSAA had a significantly higher survival rate (100%) compared to those fed ASPP (86.7%), AA (53.3%), or C (36.7%). Weight gain (%) and specific growth rate (SGR) of prawns fed with diets ASPP and CSAA were significantly higher than those fed with diets C and AA. Whole Body Ascorbic Acid (WBAA) concentration was significantly higher in prawns, fed diets supplemented with CSAA and ASPP compared to those received diet AA and C. The results indicated that chitosan stabilised ascorbic acid in a heat-stable, water-insoluble, and bioavailable form for *M. rosenbergii* juveniles provides a comparable growth performance, WBAA content, and better survival compared to the commercial stabilised ascorbic acid formulation: ascorbyl-2-polyphosphate.

Keywords: Ascorbic acid, Vitamin C, Feed evaluation, Freshwater prawn, *Macrobrachium rosenbergii*, Whole-body ascorbic acid.

INTRODUCTION

Aquatic animals, especially fish and prawns, cannot synthesize ascorbic acid as they lack the enzyme L-gulonolactone oxidase. Ascorbic acid is essential for normal growth, immunity, and reproduction in finfish and shellfish (Lovel, 1973; Wilson and Poe, 1973; Desimaru and Kuroki, 1976; Hilton *et al*., 1978; Margarelli *et al*., 1979; Shiau and Jan, 1992; Lee *et al*., 1998, Shahkar, 2015; Asaikkutti *et al*., 2016; Zafar and Khan, 2020; Yusuf *et al*., 2020). L-ascorbic acid is extremely labile and its chemistry suggests that the rate of degradation can be a function of storage time, temperature, oxygen, pH, and light (Herreid *et al*., 1952; Wanninger, 1972; Lim and Lovell, 1978; Soliman, *et al*., 1987). The degradation of L-ascorbic acid loss can be reduced to some extent by polymer or silicon coating (Skelbnaek *et al*., 1990; Moreau *et al*., 1998) encapsulation with ethylcellulose or other impervious materials (Adams, 1978; Robinson, 1992). The reactive component of ascorbic acid is the hydroxyl group (OH) at the C2 position; it is possible to protect this group through esterification by replacing it with a sulphate or phosphate group. Since these derivatives of ascorbic acid are relatively resistant to degradation through heat and oxidation (Shigueno and Itoh, 1988; Grant *et al*., 1989; NRC, 1993) it would be advantageous to use them in fish and prawn feeds.

Phosphate derivatives of ascorbic acid have been shown to have antiscorbutic activity in channel catfish (Wilson *et al*., 1989; El Naggar and Lovel, 1991), tilapia (Soliman *et al*., 1986), rainbow trout (Cho and Cowey, 1993; Dabrowski *et al*., 1996), Japanese yellowtail (Kanzawa *et al*., 1992), *Marsupenaeus japonicus* (Shigueno and Itoh, 1988), *Penaeus monodon* (Shiau and Hsu,1994), *Macrobrachium rosenbergii* (Hari and Kurup, 2002), *Marsupenaeus japonicus* (Moe et *al*., 2005), *Litopenaeus vannamei* (Niu *et al*., 2009; Chen et al., 2017) and to have been utilised successfully to enrich *Artemia* for use in live larval feeds (Smith *et al*., 2004 a, b). Matusiewicz and Dabrowski (1995) reported that the alkaline phosphatase which is seen as abundant in the intestine of rainbow trout serves to hydrolyase the ascorbate phosphate releasing active ascorbic acid into the circulation. However, the production of the polyphosphate and monophosphate esters of ascorbic acid requires extensive purification, a process that increases the cost of production (Liao and Seib, 1990). It is therefore of interest to feed manufacturers to use cheaper ascorbate derivatives with high stability while still remaining bioavailability to fish and prawns.

Several attempts have been made to develop a more stable form of ascorbic acid for aquaculture feeds. The ascorbic acid in the present composition was stabilised through the use of the cationic polymer chitosan. The

chitosan-stabilised ascorbic acid was synthesised by the Central Institute of Fisheries Technology, Cochin, India. Before any compound can be used to replace traditional forms of stabilised ascorbic acid, in aquatic feeds information on efficacy must be attained (Lovell and El Naggar, 1989). Therefore, during this study the efficacy of chitosan stabilised ascorbic acid has been compared with the commercially available ascorbic acid ascorbyl-2-polyphosphate (ROVIMIX Stay – C 35, Hoffman La Roche Vitamins, NJ., USA) and crystalline L-ascorbic acid (Qualigens Fine Chemicals, Mumbai, India) on the growth performance, survival and whole-body ascorbic acid content (WBAA) of *M. rosenbergii* juvenile. Furthermore, investigations were also made on the effect of feed processing, leaching, and storage on dietary retention of the above-said ascorbic acid sources.

MATERIAL AND METHODS

Experimental diets

The composition of the basal semi-purified control diet (C) devoid of ascorbic acid is shown in Table 1. Three semi-purified diets were prepared using the following ascorbic acid sources: chitosan stabilised ascorbic acid (CSAA), ascorbyl-2-polyphosphate (ASPP), crystalline L-ascorbic acid (AA), diets were formulated to contain 250 mg ascorbic acid equivalents (AAE) kg^{-1} diet. The CSAA and ASPP are reported to contain 50% and 35% ascorbic acid activity, respectively. A vitamin premix without ascorbic acid was used in all experimental diets. Vitamin-free casein (Himedia Laboratories, Mumbai, India) was the protein source used in all experimental feeds. In diets supplemented with ascorbic acid the equivalent amount of alpha cellulose was removed. The experimental diets were formulated to contain 400 g crude protein kg^{-1} diet and 70 g crude lipid kg^{-1} diet. The ingredients were weighed according to the formula (Table 1) and mixed thoroughly. Approximately 400 ml warm water (60 ºC) was added per kilogram diet, mixed thoroughly and the dough was passed through a hand pelletizer using a 2 mm die. The resultant pellets were steamed for 3 min. and dried in a hot air oven at 50ºC for 2 hrs. and followed by fan drying at room temperature (28 ºC) for 2 hrs. The dry pellets were broken into pieces of 2-3 mm and stored in airtight plastic containers at -18 ºC until use.

Experimental animals and feeding trial

Twenty-day-old post-larvae of *Macrobrachuim rosenbergii* (de Man) reared from a single brood at the Hatchery Complex of the School of Industrial Fisheries were nursed for 30 days in 1000 L fibre-reinforced plastic (FRP) tanks. An ascorbic acid-free basal control diet (C) was given to the prawns for two weeks during the second half of the nursery rearing to acclimatise them to the experimental diets and deplete the body stores of ascorbic acid. A total of 120 uniformly sized prawns with a mean ± SD weight of 64 ±0.02 mg were distributed in the four dietary treatments (30 individuals per treatment). The post larvae were visually checked for signs of diseases or parasites, but none were observed. Round aquamarine coloured 120 L FRP tanks with a 0.4 m^2 tank base was filled with 100 L of de-chlorinated tap water. Triplicate tanks were maintained for each treatment (10 animals in each culture tank) and the tank allocation for each treatment was completely randomised. Water was exchanged 50% daily at 09.00 hours and a 12 h light and 12 h dark photoperiod was maintained throughout the experimental period. Pelleted sinking experimental diets were given to the experimental animals at 120 g kg^{-1} day^{-1} of initial weight and after the first week, it was then adjusted to 70 to 80 g kg^{-1} day^{-1}. The daily ration was divided into two equal feeds, which were distributed evenly over the tank's surface, at 10.00 and 18.00 hours. After 5 hrs., the unconsumed feed was removed, and dried and the weight was recorded. Records were maintained for the feed applied and unconsumed. The experimental tanks were scrubbed on alternate days to minimise fungal and algal growth that could provide a source of ascorbic acid to the experimental animals. Survival was recorded through daily counts. On day 50 of the culture, the feeding experiment was terminated and prawns were harvested after draining the tanks.

Sample collection and analysis

The initial and final live weight of the prawns was measured individually using an electronic balance after blotting with soft tissue paper. Body length was measured using a dial caliper. The test animals were observed for physical abnormalities. Specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival were calculated as follows:

SGR = [(ln final weight -ln initial weight) x 100]/ days of experiment

FCR= feed consumed (dry weight)/ live weight gain (wet weight)

PER= live weight gain (g)/ protein consumed (g)

Survival rate (%) = 100 – (Initial No. of prawns - Final No. of prawns) x 100/ Initial No. of prawns

Daily water temperature and pH were measured using a mercury thermometer and a pH meter, respectively. Dissolved oxygen and ammonia were determined following the standard procedures (APHA, 1995). Moisture, crude protein, and crude fibre were determined following AOAC (1990). Crude lipid was estimated by soxhlet extraction with petroleum ether (BP 60–80 ºC) and the ash content was determined from the residue remaining after incineration of the samples for 24 hrs at 550ºC in a muffle furnace. The nitrogen-free extracts (NFE) were computed by difference (Crompton and Harris, 1969).

The ascorbyl-2-polyphosphate content of the diet ASPP was assayed following Wang *et al*. (1988). The total ascorbate content of the other experimental diets was assayed following (Roe, 1967). Leaching loss of the ascorbic acid sources was determined by following Soliman *et al*. (1987) at 28 ºC water temperature for 15 and 30 min. Storage studies were conducted following Soliman *et al*. (1987) and samples from each diet were stored at room temperature (27 to 29ºC) in black plastic

bags. The ascorbic acid content of the experimental diets after processing, leaching, and storage was expressed as the retention (%).

At the termination of feeding trials, prawns were weighed and samples were prepared by homogenising in 5 ml of ice-cold 5% trichloroacetic acid (TCA) for the analysis of whole-body ascorbic acid (WBAA) content of the prawns. The homogenates were centrifuged for 20 min. at 3000 rpm. The ascorbic acid content of the supernatant was analysed using the DNPH method described by Omaye *et al*. (1979) and expressed as $\mu g.g^{-1}$ wet body weight of prawn.

Statistical analysis

Variables such as prawn growth, FCR, SGR, and PER, survival, the ascorbic acid content in experimental diets, and ascorbic acid retention (%) after processing, leaching, storage, and WBAA content of prawns were analysed by one-way ANOVA. All percentage data were normalised by square root arc-sign, but only non-transformed data are presented. Water quality parameters were subjected to split-plot ANOVA. All the ANOVA were performed using the SPSS 11 program for Windows. All effects were considered significant at a level of significance of $P<0.05$. Tukey's test was applied for post-hoc comparison.

RESULTS AND DISCUSSION

Water temperature, pH, dissolved oxygen and ammonia ranged from 27 to 29 ^{o}C, 7.2 to 7.8, 6.1 to 7.2 mg L^{-1}, and <0.01 mg L^{-1}, respectively throughout the experimental period and were not significantly different between treatments. The proximate composition and ascorbic acid content (mg AAE kg^{-1} diet) of the experimental diets are given in Table 2. The significantly lower growth performance, survival rate, and protein conversion efficiency of *M. rosenbergii* juvenile-fed diet AA indicated that crystalline L-ascorbic acid at 250 mg AAE kg^{-1} diet inclusion concentration could not satisfy the ascorbic acid requirement of *M. rosenbergii*. This was due to its lower stability and only 42% of the crystalline L-ascorbic acid was retained in the feed after processing. Soliman *et al*. (1987) reported retention of only 38% L-ascorbic acid in the diet after feed processing. Chitosan stabilised ascorbic acid and ascorbyl-2-polyphosphate content in experimental diets was not significantly affected by feed processing.

Retention (%) of various ascorbic acid sources after processing, leaching, and storage in experimental diets are shown in Table 3. Feed processing including mixing, the addition of warm water (60 ^{o}C), pelleting, and drying at 50^{o}C had a negligible effect on the stability of chitosan stabilised ascorbic acid and ascorbyl-2-polyphosphate. However, significantly low L-ascorbic acid recovery was recorded in diet AA after feed processing. Gadient and Schai (1994) and Moreau *et al.* (1998) recorded 98% and 86-100% retention of ascorbyl-2-polyphosphate in shrimp feeds after processing. Grant *et al*. (1989) reported that ascorbyl-2-polyphosphate is a water and heat-stable ascorbic acid derivative with stability in pelleted feeds reported to be up to 83 to 45-times greater than that of crystalline L-ascorbic acid at 25 and 40 ^{o}C, respectively. The results of the present study revealed that chitosan stabilised ascorbic acid had similar ascorbic acid retention to ascorbyl-2-polyphosphate after feed processing.

Increasing immersion time led to increased leaching of ascorbic acid. Significantly higher ascorbic acid retention was recorded after 30 min. of leaching in diets supplemented with ascorbyl-2-polyphosphate and chitosan-stabilised ascorbic acid compared to L-ascorbic acid. Shiau and Hsu (1994) reported that even at relatively mild conditions of feed preparation in the lab compared to those used in feed manufacturing, the process destroyed approximately 67 to 75% of crystalline L-ascorbic acid in the feed and a reduction of activity up to 85.7% after one-hour immersion in water prior to analysis was also recorded. Aquatic crustaceans, unlike fish, are slow feeders, and food particles usually remain suspended in water for extended periods before being consumed. Increased leaching may also be due to external manipulation of the diet by the prawns during feeding. Only 57% of the initial dietary content of L-ascorbic acid content was retained after 30 min. of the leaching experiment. Soliman *et al*. (1987) reported only 43% retention of L-ascorbic acid after 3 min. of exposure to water at 28 ^{o}C. However, Khajarern and Khajarern (1997) recorded 79.9% L-ascorbic acid retention in catfish feed after 30 min exposure to water. Ascorbyl-2-polyphosphate and chitosan stabilised ascorbic acid recorded 90% and 91% retention, respectively after 30 min. leaching. This agrees with Moreau *et al*. (1998) who recorded a dietary retention of 93 to 91% ascorbyl-2-polyphosphate after 15 to 30 minutes of leaching.

After 2 months of storage of the experimental diets, retention (%) of L-ascorbic acid was significantly affected compared to ascorbyl-2-polyphosphate and chitosan stabilised ascorbic acid. There was no significant difference in the retention (%) ascorbic acid after 2 months of storage at 28 ^{o}C between diets supplemented with chitosan stabilised ascorbic acid and ascorbyl-2-polyphosphate. Volker and Fenster (1994) recorded 55% L-crystalline ascorbic acid retention in rainbow trout feeds after 8 weeks of storage. While it was only 25% in catfish feed after 2 months of storage (Khajarern and Khajarern, 1997). The use of crystalline L-ascorbic acid necessitates a higher level of inclusion in crustacean diets compared to phosphate derivatives of ascorbic acid (Shiau and Hsu, 1994; D' Abramo, *et al*., 1994) increasing the cost of the diet through the higher level of inclusion. Grant *et al*. (1989) reported a retention of 84% and 65% ascorbic acid activity in the ascorbyl-2-polyphosphate supplemented feed after steam pelleting and storage of 60 and 90 days, respectively at 40 ^{o}C. Retention of 86 to 88% ascorbic activities after 2 months of storage at room temperature would suggest that ascorbyl-2-polyphosphate and chitosan stabilised ascorbic acid are stable forms of

ascorbic acid which can be used as ascorbate sources in aquaculture feeds.

Loss of ASPP and CSAA demonstrated during the feed processing was only minor and overall retention was 97% for both the ascorbic acid sources. Gadient and Schai (1994) showed similar retention in the range of 91 and 98% for ascorbyl-2-monophosphate and ascorbyl-2- polyphosphate, respectively in pelleted shrimp feed. Lavens *et al*. (1999) recorded retention of 99% of ascorbyl-2-monophosphate in purified micro-bound diets for *Litopenaeus vannamei*. Moreover, 86-100% ascorbic acid retention was recorded by Moreau *et al.* (1998) in practical diets of *P. monodon*. In the present investigation, comparable water retention of ascorbic acid derivatives was recorded in CSAA (89%) and ASPP (87%) and the loss of ascorbic acid potency was negligible after 30 minutes of exposure to water, the period of time required by the prawns to complete a meal. Thus, by using CSAA it is feasible to quantify ascorbic acid intake by *M. rosenbergii* and other prawns because of its comparable stability and residence in the pelleted feed with that of ASPP.

Results of the survival, growth performance, FCR, and PER are summarized in Table 4. The survival of the prawns was significantly affected by the dietary treatments. Survival was significantly higher for prawns receiving the dietary treatment CSAA compared to those fed ASPP with subsequent lower survival in prawns fed AA and C diets, respectively. After 50 days of feeding the experimental diets to the prawns their growth performance was significantly affected. The percentage increase in body length was significantly higher for prawns receiving diets CSAA and ASPP compared to those fed diets AA and C. The prawns consuming diets CSAA and ASPP showed significantly higher weight gain (%) compared to those fed diets AA and C. There was no significant difference in weight gain (%) between prawns fed diet CSAA and diet ASPP. SGR also showed the same trend. Comparable weight gain, SGR, and improved survival of juvenile *M. rosenbergii*, fed a chitosan stabilised ascorbic acid diet demonstrated the efficacy of this form of ascorbic acid; producing similar results to ascorbyl-2-polyphosphate for use in this life-stage of *M. rosenbergii*. Ascorbic acid plays an important role in certain aspects of protein metabolism (Chatterjee, 1967) and has a specific effect on fish growth (Ram, 1966).

The FCR, and PER values for diets CSAA and ASPP were not significantly different. In the present study, prawns fed the CSAA and ASPP diets attained significantly (P<0.05) lower FCR compared to prawns fed diets devoid of ascorbic acid. Prawns fed diets CSAA and ASPP recorded significantly higher PER values compared to diets AA and C. This suggests that *M. rosenbergii* utilized CSAA as a source of ascorbic acid similar to ASPP, and may have assisted in the utilization of dietary protein. Dietary supplementation of stable ascorbic acid derivatives such as L-ascorbyl-2-monophosphate, L-ascorbyl-2-polyphosphate, L-ascorbyl-2-monophosphate-Na/Ca positively influenced the feed and protein conversion efficiency in *P. monodon* (Shiau and Hsu, 1994), olive flonder, *Paralichthyus oliaceus* (Wang *et al*., 2002) and Korean rockfish, *Sebastes schlegelii* (Wang *et al*., 2003).

There were considerable benefits noted in the use of chitosan stabilised ascorbic acid at a level of 250 AAE kg^{-1} diet, no gross signs of ascorbic acid deficiency were observed in prawns fed this prepared diet (Fig.1). This supports the suggestion that chitosan stabilised ascorbic acid is biologically active for juvenile *M. rosenbergii* enabling them to meet their antiscorbutic vitamin requirements. Significantly higher survival was recorded for the *M. rosenbergii* juveniles fed CSAA diets compared to all other dietary treatments. Feeding the diets devoid of ascorbic acid to prawns resulted in significantly lower growth performance in terms of length increase, weight gain, and a lower PER compared to either diets supplemented with CSAA or ASPP. Results of the present study showed that stable dietary ascorbic acid sources such as chitosan stabilised ascorbic acid and ascorbyl-2-polyphosphate at a dietary level of 250 mg AAE kg^{-1} diet had significant effect on the growth and FCR of *M. rosenbergii* juveniles. Hari and Kurup (2002) recorded significantly greater body length and weight gain in *M. rosenbergii* fed 150 and 250 mg AAE kg^{-1}diet of ascorbyl-2-polyphosphate compared to animals fed at a zero level of inclusion. Lavens *et al*. (1999) reported significantly higher growth in *L. vannamei* receiving 100 to 200 mg AAE kg^{-1} of ascorbyl-2-polyphosphate compared with those fed 0-10 mg AAE kg^{-1}. Similarly, Shiau and Hsu (1994) reported that *P. monodon* fed diets supplemented with ascorbyl-2-polyphosphate (30 to 2000 mg kg^{-1} diet) had significantly higher weight gain and lower FCR than those fed an unsupplemented control diet. *M. malcolmsonii* fed a diet supplemented with 25–100 mg/kg of vitamins C showed enhanced (P<0.05) growth performance, including final weight and weight gain (WG) (Asaikkutti *et al*., 2016). However, D' Abramo (1994) suggested that weight gain in *M. rosenbergii* was not significantly affected by varying dietary levels of ascorbyl-2-monophosphate Ca salt and ascorbyl-6-palmitate. Similar results were recorded in *Litopenaeus vannamei* in an 8-week feeding trial which was conducted to evaluate the effects of ascorbic acid (AsA), in the form of L-ascorbyl-2-polyphosphate (LAPP) (Niu *et al*., 2009; Chen *et al*., 2017).

Ascorbyl-2-polyphosphate is utilised extensively in the aquaculture feed industry. It has proven to be a good dietary source of ascorbic acid in many species including *P. monodon* (Shiau and Hsu, 1994; Moreau *et al*., 1998) channel catfish (Wilson *et al*., 1989) trout and fathered minnow (Grant *et al*., 1989). The significant improvement in the survival of prawns by adding ASPP to their diets indicated that ASPP is also an effective dietary source of ascorbic acid. The results of the present study confirmed the results of the previous observations of Hari and Kurup (2002) that ascorbyl-2-

polyphosphate can be effectively utilised as a dietary source of ascorbic acid for *M. rosenbergii*.

In the present study, a high mortality rate was recorded in *M. rosenbergii* juveniles fed the diet supplemented with crystalline L-ascorbic acid and the diet devoid of ascorbic acid, and during the third and fourth weeks of the experiment, it was primarily associated with a moulting event, with many dead prawns displaying exuvia-entrapment. D' Abramo *et al*. (1994) and Hari and Kurup (2002) made similar observations in *M. rosenbergii* juveniles. The inability to moult completely when fed an ascorbic acid-deficient diet has been reported for *M. japonicus* (Desimaru and Kuroki 1976) *Litopenaeus vannamei* (He and Lawrence, 1993). Lightner *et al*. (1977) reported the inability of ascorbic acid-deficient shrimp to hydroxylate sufficient procollagen to produce mature collagen fibres. Similarly, Moreau *et al.* (1998) recorded that hepatopancreas ascorbic acid and collagen content were significantly lower in *P. monodon* deprived of ascorbic acid. In the present study, the impaired moulting performance observed in prawns fed a diet supplemented with crystalline L-ascorbic acid or totally devoid of ascorbic acid may be attributed to the aforesaid reasons and are commonly termed 'incomplete moulting' (Chen & Chang, 1994) or 'inability to extricate successfully from the old exoskeleton during ecdysis' (D' Abramo, *et al*., 1994). Boonyaratpalin and Phongmaneerat (1995) and Reddy *et al*. (1999) reported that *P. monodon* fed ascorbic acid-deficient diets showed poor food intake, anorexia, and prolonged deficiency results in blackening of gills and lesions in the abdominal region.

The WBAA concentrations of the experimental prawns are shown in Fig. 1. The dietary treatments significantly influenced the WBAA of *M. rosenbergii* juveniles. Significantly higher WBAA concentrations were recorded for prawns consuming diets CSAA and ASPP, which did not differ, compared to the WBAA concentration of prawns fed diets AA and C. After 50 days of feeding the formulated diets, the WBAA concentration of prawns fed diet CSAA and diet ASPP were significantly greater than their initial concentrations. The lowest WBAA concentration was recorded in prawns that, consumed diet C. Moreover, the prawns fed diets AA and C failed to accumulate ascorbic acid during the study with WBAA concentrations significantly less than their initial concentrations. Hari and Kurup (2002) reported that juveniles of *M. rosenbergii* appeared to require a minimum of 135 mg AAE kg^{-1} diet to maintain normal survival. WBAA content of about 8.5 ug g^{-1} was required to prevent the occurrence of high mortality and values below this level may indicate ascorbic acid deficiency in *M. rosenbergii* and relatively low depletion levels for WBAA from 2.27 to 7.61 ug g^{-1} were observed in prawns fed diets containing ascorby-2-polyphosphate from 0 to 100 mg AAE kg^{-1} (Hari and Kurup, 2002). In the present investigation, diet deficient in AA and in crystalline L-ascorbic acid dietary treatments, the WBAA was significantly lower than that found essential for normal survival as reported by Hari and Kurup (2002). This may be the reason for lower survival in a diet devoid of ascorbic acid and a diet supplemented with crystalline L-ascorbic acid. A dietary requirement of 120 mg AAE kg^{-1} diet was reported in *L. vannamei* having a body weight of 0.1 g for maintaining normal survival and WBAA content (He and Lawrence, 1993).

In ascorbic acid-supplemented diets, *P. monodon* (Shiau and Hsu, 1994) and *L. vannamei* (He and Lawrence, 1993) survival were significantly higher than the non-supplemented diets. Furthermore, survival of *L. vannaemi* (0.1g mean body weight) fed graded levels of ascorbyl-2-polyphosphate (0-400 mg AAE kg^{-1} diet) showed a significant difference in their survival rate (He and Lawrence, 1993). In the present study, prawn fed a diet containing CSAA showed significantly higher survival compared to ASPP while, in *P. monodon* fed a diet contacting ascorbyl-2-polyphosphate and silicon-coated ascorbic acid there exists no significant difference in survival at two dietary levels (500 and 1000 AAE kg^{-1}) (Moreau *et al*., 1998). Survival of *M. rosenbergii* fed diet contacting ascorbyl-2-monophosphate and ascorbyl-6-palmitate (AP) at graded levels regardless of the source $\geq$50 mg AAE kg^{-1} diet was $\geq$75% survival rate. In the present study dietary treatments containing CSAA and ASPP at 243 and 242 mg AAE kg^{-1}, respectively showed significantly higher survival >75% as reported by D'Abramo (1996). *Marsupenaeus japonicus* fed diets with AsA 0, 20 and 56 mg kg^{-1} showed high cumulative mortality after 10 days of feeding (Moe, *et al*., 2005).

The WBAA levels of the *M. rosenbergii* juvenile fed diets CSAA and ASPP are not significantly different indicating that CSAA is stable, biologically active, and plays an important role in the growth and survival of prawns. After the 50 days of the current study, the WBAA concentration in prawns fed diets AA and C was less than the initial depleted WBAA content. This suggests that diet AA containing only 105 mg AAE kg^{-1} diet of L-ascorbic acid failed to provide sufficient WBAA levels necessary to prevent ascorbic acid deficiency signs due to low stability during feed processing, leaching, and storage (Hilton *et al*., 1977; Soliman *et al*., 1987; Gadient and Schai, 1994). Hari and Kurup (2002) recommended a concentration of 135 mg AAE kg^{-1} diet for juvenile *M. rosenbergii* as a dietary requirement to maintain a WBAA necessary to provide good growth performance, feed conversion, and survival. Lightner *et al*. (1977) and He and Lawrence (1993) suggested that crustaceans have limited ability for *de novo* synthesis of ascorbic acid. The results of the present study revealed that, in *M. rosenbergii* juvenile with a body weight of 64 mg this ability was not sufficient to meet their ascorbic acid requirements and suggest that their diets have to be supplemented with stable forms of ascorbic acid.

WBAA content of the prawns were significantly reduced by feeding ascorbic acid deficient diets when

compared to diets supplemented with ASPP and CSAA. Similar results have been demonstrated by in *P. monodon* (He and Lawrence, 1993); in channel catfishes (Wilson *et al*., 1989), in trout (Grant *et al*., 1989). In the present study, after a depletion period of 14 days, the estimated initial WBAA content of *M. rosenbergii* juveniles (4.0 µg g^{-1}) was further reduced to 2.3 µg g^{-1} due to continued feeding with the ascorbic acid deficient practical diet for 50 days during the experiment. Similarly, a reduction of hepatopancreatic ascorbic acid level from 27.9 µg g^{-1} to 1.35 µg g^{-1} was recorded in *P. monodon* (mean body weight 0.9) on a diet lacking ascorbic acid for 5 weeks and a further reduction of 1.27µg g^{-1} was also recorded during the 13 weeks of experimental period (Moreau *et al*., 1998). Hari and Kurup (2002) recorded a WBAA content of 2.27 and 3.89 µg g^{-1} in *M. rosenbergii* fed ascorbic acid-free purified diet and a diet supplemented with 50 mg AAE Kg^{-1} diet of ascorbyl-2-poly phosphate in a 7-week feeding experiment. The WBAA ranged from 2.6 to 3.0 µg g^{-1} in *L. vannamei* juveniles having 0.1g body weight fed an ascorbate deficient diet purified diet (He and Lawrence, 1993).

Prawns fed the diet containing L-crystalline ascorbic acid had a WBAA content that was approximately 39% of the WBAA content found in prawns fed diets ASPP and CSAA. This was in accordance with the earlier reports of Wilson *et al.* (1989) who recorded the carcass ascorbate concentration of channel catfish fed coated form of crystalline L-crystalline ascorbic acid contain only 40% of the total ascorbate concentration found in fish fed diets containing ascorbyl-2-polyphosphate. The WBAA content of prawns feed diet containing crystalline L-ascorbic acid was only 4.2 µg g^{-1} and it revealed that it did not significantly improve the WBAA from the initial content (4.0 µg g^{-1}) after 50 days of feeding trial. Low WBAA content of the dietary treatment AA can be attributed to the leaching of hydrophilic L-crystalline ascorbic acid in the feed when exposed to water causes substantial losses of ascorbic acid before the pellet was ingested by the prawn. The results of the present study revealed that 250 mg AAE kg^{-1} crystalline L-ascorbic acid added to the diet before feed processing was reduced to 105 mg AAE kg^{-1} due to pelletization process after immersion in water for 30 min it was further reduced to 61 mg kg^{-1} diet which indicted that only 24.4% retention of the dietary supplementation was available to the prawns for ingestion. Similarly, Gadient and Sachai (1994) demonstrated only 20 to 30% retention of crystalline L-ascorbic acid in shrimp immersed for 2 hr. in water. Furthermore, He and Lawrence (1993) reported that dietary supplementation of ascorbic acid from 0-90 mg AAE kg^{-1} might result only slight increase in the WBAA (2.6 to 3.0 µg g-1) in *L. vannamei*. Ascorbyl-2-polyphosphate at a dosage of 50 and 100 mg AAE kg^{-1} in the diets of *M. rosenbergii* recorded significantly lower WBAA compared to 250 mg AAE kg^{-1} (Hari and Kurup, 2002). D'Abramo (1994) used very high dietary concentrations of crystalline ascorbic acid (5000 mg kg^{-1} diet) in a semi-purified basal diet to ensure comparable growth and survival in *M. rosnebergii* with ascorbyl-2-polyphosphate.

Table 1: Inclusion of feed ingredients in the experimental basal diet (g kg^{-1}).

Casein (vitamin free)	350
Starch	230
Lipid mix [a]	70
Aminoacid mix [b]	30
Dextrin	50
Gelatin	50
Sodium citrate	3
Mineral and vitamin mix [c]	20
Choline chloride	10
Chitosan	8
Ascorbic acid	0
Cholesterol	5
Carboxy methyl cellulose	10
Alpha cellulose	164

[a] Lipid Mix -1:1 ratio of Cod liver oil and Sunflower oil

[b] Amino acid Mix - 1:1 ratio of Glycine and Betaine

[c] Mineral and vitamin mix (g kg^{-1})

Vitamin A 62 500 IU; Vitamin D_3 30000 IU; Vitamin E 0.05; niacinamide 0.225;

thiamine mononitrate, 0.3; riboflavin, 3.0; folic acid, 0.5; biotin, 0.1; choline chloride, 100.0

pyridoxine HCL 1.0; D pantothenate 3.75; cyanocobalamine 0.05;

K_2HPO_4 100; $NaH_2PO_4.H_2O$ 215; $Ca(H_2PO_4)$ 265, $CaCO_3$ 105; Ca lactate 165;

KCl 28; $MgSO_4$. 7 H_2O 100; Fe citrate 12; $ZnSO_4$ 0.5; KI 1.0; $CoCl_2$. 6 H_2O 0.1;

$CuCl_2$ 0.5; SeO_2 0.05; $MnSO_4$ 1.0; $AlCl_3$ 0.5.

[d] 250 mg ascorbic acid equivalent (AAE) kg^{-1} of chitosan stabilized vitamin C; ascorbyl -2-polyphosphate; crystalline ascorbic acid

Table 2: Proximate composition (g kg^{-1}) of experimental diets.

	Diet C	Diet AA	Diet ASPP	Diet CSAA
Protein	409.1	401.9	395.6	406.1
Lipid	77.8	70.3	71.1	70.5
Moisture	85.7	86.3	89.7	86.5
Ash	21.9	19.3	19.9	21.0
Fibre	28.7	31.2	29.9	28.3
NFE [1]	376.8	391.0	393.8	387.6

[1]NFE: Nitrogen Free Extract

Table 3: Ascorbic acid content (mg AAE Kg^{-1}) and retention (%) in diets after processing, leaching and storage
[Mean values ± S.D. with different superscript vary significantly (P< 0.05)].

	Diet C	Diet AA	Diet CSAA	Diet ASPP
After feed processing*	N.D.[1]	105.4±1.3^{b}	242.9±2.7^{a}	241.9±1.8^{a}
Retention (%) after feed processing**	N.D.[1]	42±0.9^{b}	97±1.1^{a}	97±0.7^{a}
After 15 min. leaching	N.D.[1]	71.1±0.9^{b}	225.3±3.8^{a}	224.3±3.5^{a}
Retention (%) after 15 min. leaching [#]	N.D.[1]	68±0.9^{b}	93±1.3^{a}	93±0.9^{a}
After 30 min. leaching	N.D.[1]	60.±3.2^{b}	221.7±5.6^{a}	218.5±3.8^{a}
Retention (%) after 30 min. leaching[#]	N.D.[1]	57±2.5^{b}	91±1.4^{a}	90±0.9^{a}
After one month storage [2]	N.D.[1]	44.8±1.2^{b}	217.1±2.6^{a}	210.4±2.5^{a}
Retention (%) after one month storage [2 #]	N.D.[1]	43±0.7^{c}	89±0.7^{a}	87±0.5^{b}
After 2 months storage[2]	N.D.[1]	29.8±0.1^{b}	212.8±3.1^{a}	207.1±4.4^{b}
Retention (%) after 2 months storage[2 #]	N.D.[1]	28±0.3^{b}	88±1.4^{a}	86±1.5^{a}

[1]ND: None detected
[2] Storage in sealed black plastic bags at 27 to 29 ^{o}C
* Initial ascorbic acid content in feed (mg AAE Kg-1) used for leaching and storage studies
** Percentage of initial concentration of ascorbic acid sources in the feed material (250 mg AAE Kg^{-1}) before processing
[#] Percentage of initial concentration of ascorbic acid sources in the feed before leaching and storage

Table 4: Length (cm) and weight gain (g), percentage length (%) and weight gain (%), SGR[1], FCR[2], PER[3] and survival (%) of juveniles of *Macrobrachium rosenbergii* fed diets containing different Ascorbic acid sources
[Mean values with different superscript vary significantly (P< 0.05); Values are expressed as mean of triplicates ± S.D.]

	Control	Diet AA	Diet ASPP	diet CSAA	Significance[†] P- value
Initial length	2.4±0.1	2.5±0.1	2.3±0.1	2.5±0.1	
Final length	2.9±0.1	3.04±0.1	3.19±0.2	3.39±0.1	
Length increase	0.47±0.1^{b}	0.50±0.1^{b}	0.85±0.1^{a}	0.92±0.1^{a}	**
Length increase (%)	19.2±3.4^{b}	19.6±5.7^{b}	36.3±2.5^{a}	37.3±5.6^{a}	**
Initial weight	0.067±0.003	0.063±0.001	0.065±0.001	0.066±0.002	
Final weight	0.138±0.004	0.147±0.006	0.209±0.002	0.222±0.02	
Weight gain	0.07±0.004^{b}	0.08±0.005^{b}	0.14±0.002^{a}	0.16±0.016^{a}	***
Weight gain (%)	113.0±8.7^{b}	134.0±7.5^{b}	223.8±4.7^{a}	238.4±20.5^{a}	***
SGR[1]	1.5±0.1^{b}	1.7±0.1^{b}	2.4±0.03^{a}	2.4±0.1^{a}	***
FCR[2]	3.1±0.2^{a}	2.7±0.2^{b}	1.8±0.03^{c}	1.6±0.2^{c}	***
PER[3]	0.8±0.1^{b}	0.9±0.1^{b}	1.4±0.02^{a}	1.5±0.2^{a}	***
Survival (%)	36.7±5.8^{d}	53.3±5.8^{c}	86.7±5.8^{b}	100.0±0.0^{a}	***

[1] Specific Growth Rate
[2] Feed Conversion Ratio
[3] Protein Efficiency Ratio
[†] Results of one-way ANOVA
** (P<0.01)
*** (P<0.001)

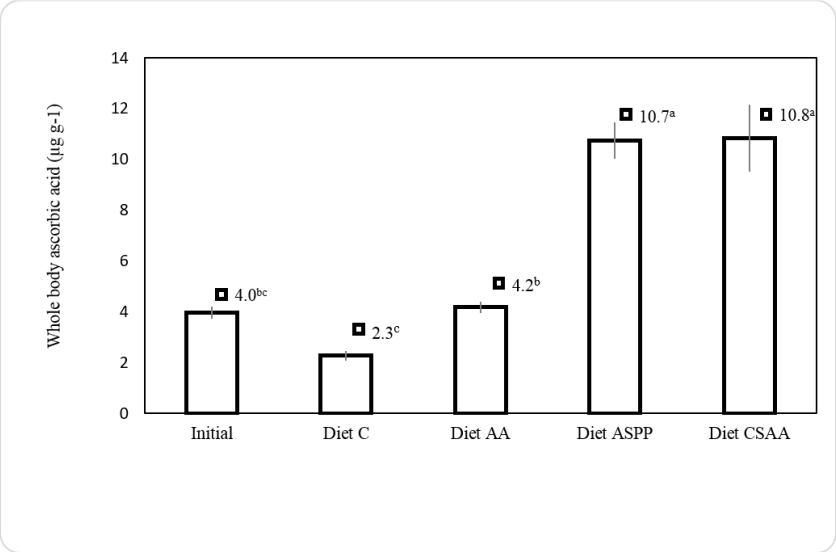

Fig. 1. The effect of diets containing various ascorbic acid sources on the whole body ascorbic acid concentration of *Macrobrachium rosenbergii* (de Man) juvenile reared for 50 days.

CONCLUSIONS

The dietary ascorbic acid sources CSAA and ASPP have a stronger influence on the growth and survival of *M. rosenbergii* compared to crystalline L-ascorbic acid. At the standard concentration (250 mg AAE kg^{-1} diet) used in all supplemented diets, the heat-labile and highly water-soluble nature of crystalline L-ascorbic acid resulted in low survival, low WBAA concentrations, and poor growth performance when compared to CSAA and ASPP supplemented diets. Under the present experimental conditions, we conclude that chitosan stabilised ascorbic acid developed by the Central Institute of Fisheries Technology, Cochin, India is heat stable, water-insoluble, bio-available and has an antiscorbutic activity similar to ascorbyl-2-polyphosphate in the diets for *M. rosenbergii* juveniles.

FUTURE SCOPE

The efficacy of chitosan stabilised ascorbic acid can be compared with the other commercially available ascorbic acids on the growth performance, survival and whole-body ascorbic acid content (WBAA) of other shellfishes of aquaculture importance. Furthermore, the investigations can be focused on the effect of different feed processing methods, and storage conditions on dietary retention of the chitosan stabilised ascorbic acid.

Conflict of Interest. Authors have declared that no competing interests exist.

Author contributions. First author conceived and designed the work. First author conducted the experiment, performed data analysis, conducted statistical tests, interpreted the results, and prepared the original draft. First and Second authors contributed to the editing and revising and improving the manuscript to the present condition. Second author also contributed to the visualisation of elements like tables and figures.

Acknowledgement. First author is grateful to the Director, School of Industrial Fisheries, Cochin University of Science and Technology, Cochin, India for providing facilities and financial assistance from Indian Council of Agricultural Research, Govt. of India. Authors are thankful to the Principal of Sree Narayana College, Nattika and Kollam for the research facilities. The authors are also acknowledging the research facility provided by DST-FIST (2018-2021) and DBT-Star College Schemes (2021-2023).

REFERENCES

A.O.A.C (1990). Official Methods of Analysis 15th edn. Association of Official Analytical Chemists, Washington, DC, USA, p.1094.

Adams, C. R. (1978). Vitamin product forms for animal feeds. In: *Proceedings of the Roche Vitamin Nutrition Conference*. RDC 5483/1078. Hoffman La Roche Inc. Nutely, N.J., 54–60.

APHA (1995). *Standard Methods for the Examination of Water and Wastewater*. 19th edn. American Public Health Association, Washington, DC, USA, p.1082.

Asaikkutti, A., Bhavan, P.S., Vimala, K., Karthik, M. and Cheruparambath, P. (2016). Effect of different levels dietary vitamin C on growth performance, muscle composition, antioxidant and enzyme activity of freshwater prawn, *Macrobrachium malcolmsonii*. *Aquaculture Reports*, 229–236. https://doi.org/10.1016/j.aqrep.2016.04.002.

Boonyartpalin, M. and Phongmaneerat, J. (1995). Ascorbic acid derivative requirement of *Penaeus monodon*. *Phuket Mar Biol Res Bull*, *60*, 65–73.

Chatterjee, G. C. (1967). Effect of ascorbic acid deficiency in animals. In: *The Vitamins* (Sebrell, W. H. R. S. ed.), Academic Press, New York, USA, *1*, 407–456.

Chen, H. Y. and Chang, C. F. (1994). Qualification of vitamin C requirements for juvenile shrimp (*Penaeus monodon*) using polyphosphorylated L-ascorbic acid. *J Nutr*, *124*, 2033–2038.

Chen, S. J., Gao, Y. J., Xie, S. X., Niu, J., Yang, F., Fang, W. P., Tian, L. X. and Liu, Y. J. (2017). Effect of L-ascorbyl-2-polyphosphate supplementation on growth performance, body composition, antioxidative capacity and salinity stress tolerance of juvenile Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture Research*, *48*(9), 4608–4622. https://doi.org/10.1111/are.13284

Cho, C. Y. and Cowey, C. B. (1993). Utilisation of monophosphate esters of ascorbic acid by rainbow

trout (*Oncorhynchus mykiss*). In: *Fish Nutrition in Practice*, Kanshik, S. J. and Luquet, P. (Eds). Paris France Institut National De La Recherche Agronomique, *61*, 149–156.

Crompton, E. W. and Harris, L. E. (1969). *Applied Animal Nutrition*, 2nd edn., Freeman, San Francisco, p.753.

D'Abramo, L. R., Moncreiff, C. A., Holcomb, F. P., Montanez, J. L. and Buddington, R. K. (1994). Vitamin C requirements of the freshwater prawn, *Macrobrachium rosenbergii*. *Aquaculture*, *128*, 269–275.

Dabrowski, K., Moreau, R. and El-Saidy, D. (1996). Ontogenetic sensitivity of channel catfish to ascorbic acid. *J Aquat Anim Health*, *8*, 22–27.

Deshimaru, O. and Kuroki, K. (1976). Studies on a purified diet for prawn. VII. Adequate dietary level of ascorbic acid and inositol. *Bull. Japn Soc Sci Fish*, *42*, 571–576.

El Naggar, G. O. and Lovell R. T. (1991). L-Ascorbyl-2-monophosphate has equal antiscorbutic activity as L-ascorbic acid but L-ascorbyl-2- sulphate is inferior for channel catfish. *J Nutr*, *121*, 1622–1626.

Gadient, M. and Schai, E. (1994). Leaching of various vitamins from shrimp feed. *Aquaculture*, *124*, 201–205.

Grant, B., Seib, P., Liao, M. and Copron, K. (1989). Polyphosphorylated L-ascorbic acid: A stable form of vitamin C for aquaculture feeds. *J World Aquacult Soc*, *20*, 143–157.

Hari, B. and Kurup B. M. (2002). Vitamin C (Ascorbyl-2-polyphosphate) requirement of fresh water prawn *Macrobrachium rosenbergii* (de Man). *Asian Fisheries Science*, *15*, 145–154.

He, H. and Lawrence, A. L. (1993). Vitamin C requirements of the shrimp *Penaeus monodon*. *Aquaculture*, *114*, 305–316.

Herried, E. O. Ruskin, B. Clark, G. L. and Parks, T. B. (1952). Ascorbic acid and riboflavin destruction and flavour development in milk exposed to sun in amber, clear, paper and ruby bottles. *Journal of Dairy Science, 35*, 772–778.

Hilton, J. W., Cho, C. Y. and Slinger, S. J. (1977). Factors affecting the stability of supplemental ascorbic acid in practical trout diet. *J Fish Res Board Can*, *34*, 683–687.

Hilton, J. W., Cho, C. Y. and Slinger, S. J. (1978). Effect of graded levels of supplemental ascorbic acid in practical diets fed to rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada*, *35*, 431–436.

Kanazawa, A., Teshima, S. I., Koshio, S., Higashi, M. and Itoh, S. (1992). Effect of L-ascorbyl-2-phosphate-Mg on the yellowtail *Seriola quinqueradiata* as a Vitamin C Source, *Nippon Suisan Gakkaishi*, *58*(2), 337–341.

Khajarern, J. and Khajarern, S. (1997). Stability of vitamin C-glucose in Clarias hybrid catfish (*Clarias gariepinus* X *Clarias macrocephalus*). *Aquaculture*, *151*, 219–224.

Lavens, P., Merchie, G., Ramos, X., Leon-Hing Kujan, A., Van Hauwaert, A., Pedrazzoli, A., Nelis, H. and De Leenheer, A. (1999). Supplementation of ascorbic acid-2-monophosphate during the early post-larval stages of the shrimp *Litopenaeus vannamei*. *Aquacult Nutr*, *5*, 205–209.

Lee, K. J. Kim, K. W. and Bai, S. C. (1998). Effects of different dietary levels of L-ascorbic acid on growth and tissue vitamin C concentration in juvenile Korean rockfish, *Sebastes schlegeli* (Hilgendorf). *Aquacul Res*, *29*, 237–244.

Liao, M. L. and Seib, P. A. (1990). A stable form of vitamin C: L-ascorbic acid-triphosphate. Synthesis, isolation and properties. *J Agric Food Chem*, *38*, 355–366.

Lightner, D. V., Colvin, I. B., Brand, C. and Donald, D. A. (1977). "Black death", a disease syndrome related to dietary deficiency of ascorbic acid. *Pro World Maricul Soc*, *8*, 611–624.

Lim, C. and Lovell, R. T. (1978). Pathology of the vitamin C deficiency syndrome in channel catfish (*Ictalurus punctatus*). *J Nutr*, *108*, 1137–1146.

Lovell, R. T. (1973). Essentiality of vitamin C in feeds for intensively fed caged channel catfish. *J Nutr*, *103*, 134–138.

Lovell, T. and El Naggar (1989). Vitamin C activity for L-ascorbic acid, L-ascorbyl-2 sulphate, and L-ascorbyl-2-phosphate Mg. for channel catfish. In: *Proc Third Int Symp on Feeding and Nutr in Fish*, pp. 159-165, Toba, Japan.

Magarelii, P. C. Jr., Hunter, B., Lightner, D. V. and Colvin, L. B. (1979). Black death: an ascorbic acid deficiency disease in Penaeid shrimps. *Comp Biochem Physiol*, *63*A, 103–108.

Matusiewicz, M. and Dabrowski, K. (1995). Characterization of ascorbyl esters hydrolysis in fish. *Comp Biochem Physiol*, *110* B, 739–745.

Moe, Y. Y., Koshio, S., Ishikawa, M., Teshima, S., Panganiban Jr, A., Thu, M., Michael, F. R. and Ren, T. (2005). Vitamin C requirement of kuruma shrimp postlarvae, *Marsupenaeus japonicus* (Bate), using L-ascorbyl-2-monophosphate-Na/Ca. *Aquaculture Research*, *336* (8), 739–745. https://doi.org/10.1111/j.1365-2109.2005.01260.x

Moreau, R., Cuzon, G. and Gabaudan, J. (1998). Efficacy of silicone-coated ascorbic acid and ascorbyl-2-polyphosphate to fast-growing tiger shrimp (*Penaeus monodon*). *Aquacult Nutr*, *4*, 23–29.

NRC (1993). Nutrient Requirements of Fish. National Research Council, National Academy Press, Washington DC. USA.

Niu, J., Tian, L. X., Liu, Y. J., Mai, K. S., Yang, H. J., Ye, C. X. and Gao, W. (2009) Nutrient values of dietary ascorbic acid (l-ascorbyl-2-polyphosphate) on growth, survival and stress tolerance of larval shrimp, *Litopenaeus vannamei*. *Aquaculture Nutrition*, *15*(2), 194–201.

Omaye, S. T., Turnbull, L. D. and Sauberlich, H. E. (1979). Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. In: *Methods in Enzymology* (D.B. Mc Cormick and Wright, L.D. Eds.), Academic Press, Inc., New York. *62*, 3–11.

Ram M. M. (1966). Growth rate and protein utilisation in vitamin C deficiency. *Indian J Med Res*, *541*, 946–970.

Reddy, H. R., Ganapathi, V., Naik, M. and Annappaswamy, T. S. (1999). Evaluation of the dietary essentiality of vitamins for *Penaeus monodon*. *Aquacult Nutr*, *5*, 267–275.

Robinson, E. (1992). Vitamin C studies with catfish: requirement, biological activity and stability. *Mississippi Agric. For. Exp. Stn. Techn. Bull*., *182*, 1–8.

Roe, J. H. (1967). In. Ascorbic acid. The Vitamins, Chemistry, Physiology, Pathology, Methods.

(Gyogry P. and Pearson W. N. eds.), Academic Press, New York, San Francisco, London, *7*, 27–49.

Shahkar, E., Yun H., Kim, D. J., Kim, S. K., Lee, B. I., Bai, S. C. (2015). Effects of dietary vitamin C levels on tissue ascorbic acid concentration, hematology, non-specific immune response and gonad histology in broodstock Japanese eel, *Anguilla japonica. Aquaculture*, *438*, 115–121

Shiau, S. Y. and Hsu, T. S. (1994). Vitamin C requirement of grass shrimp, *Penaeus monodon*, as determined with L-ascorbyl-2-monophosphate. *Aquaculture*, *122*, 347–357.

Shiau, S. Y. and Jan, F. L. (1992). Ascorbic acid requirement of grass shrimp *Penaeus monodon*. *Bull. Jap Soc Sci Fish*, *58*(2), 363.

Shigueno, K. and Itoh, S. (1988). Use of Mg-L-ascorbyl-2-phosphate as vitamin source in shrimp diets. *J World Aquacult Soc*, *19*, 69–175.

Skelbaek, T., Anderson, N. G., Winning, M. and Westergaard, S. (1990). Stability in fish feed and bioavailability to rainbow trout of two ascorbic acid forms. *Aquaculture*, *84*, 335–343.

Smith, G. G., Brown, M. R., and Ritar, A. J. (2004a). Feeding juvenile Artemia enriched with ascorbic acid improves larval survival in the spiny lobster *Jasus edwardsii*, *Aquacult Nutr*, *10*, 105–112.

Smith, G. G., Ritar, A. J., and Brown, M. R. (2004b). Uptake and metabolism of a particular form of ascorbic acid in Artemia nauplii and juveniles. *Aquacult Nutr*, *10*, 1–8.

Soliman, A. K., Jauncey, K. and Roberts, R. J. (1986). The effect of varying forms of dietary ascorbic acid in the nutrition of juvenile tilapia (*Oreochromis niloticus*). *Aquaculture*, *52*, 1–10.

Soliman, A. K., Jauncy, K. and Roberts, R. J. (1987). Stability of ascorbic acid (Vitamin C) and its forms in fish feed during processing, storage and leaching. *Aquaculture*, *60*, 73–83.

Volker, L. and Fenster, R. (1994). Efficacy of ascorbyl-2-polyphosphate in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, *124*, 213–217.

Wang X-Y, Liao, M. L., Hung, T. H. and Seib, P. A. (1998). Liquid chromatographic determination of ascorbate-2-polyphosphate in fish feeds by enzymatic release of L-ascorbate. *J Assoc Off Anal Chem*, *71*, 1158–1161.

Wang, X., Kim, K. W. and Bai, S. C. (2003). Comparison of L-ascorbyl-2-monophosphate Ca with L-ascorbyl-2-monophosphate Na/Ca on growth and tissue ascorbic acid concentrations in Korean rockfish (*Sebastes schegeli*). *Aquaculture*, *225*, 387–395.

Wang, X., Kim, K.W., and Bai, S. C. (2002). Effects of different dietary levels of L-ascorbyl–2-polyphosphate on growth and tissue vitamin C concentrations in juvenile olive flounder, *Paralichthys olivaceus* (Temminck at Schlegel), *Aquacul. Res*., *33*, 261–267.

Wanninger, L. A. Jr. (1972). Mathematical model predicts. Stability of Ascorbic acid in food products. *Food Technology*, *26*, 42–45.

Wilson, R. P. and Poe, W. E. (1973). Impaired collagen formation in the scorbutic channel catfish. *J Nutr*, *103*(9), 1359–1364.

Wilson, R. P., Poe, W. E. and Robinson, E. H. (1989). Evaluation of L-ascorbyl-2-polyphosphate (AsPP) as a dietary ascorbic acid source for channel catfish. *Aquaculture*, *81*, 129–136.

Yusuf, A., Huang, X., Chen, N., Apraku, A., Wang, W., Cornel, A. and Rahman, M. M. (2020). Impact of dietary vitamin C on plasma metabolites, antioxidant capacity and innate immunocompetence in juvenile largemouth bass, *Micropterus salmoides. Aquaculture Reports*, *17*, 10038.

Zafar, N. and Khan, M. A. (2020). Effects of dietary iron on growth, haematology, oxidative stress and hepatic ascorbic acid concentration of stinging catfish *Heteropneustes fossilis. Aquaculture*, 516, 734642.

How to cite this article: Hari B. and Jisha, S. (2023) Comparison of Chitosan Stabilised Ascorbic Acid with Ascorbyl-2-polyphosphate and Crystalline L-ascorbic Acid on Growth, Survival and Whole-body Ascorbic Acid Concentration in the Freshwater Prawn, *Macrobrachium rosenbergii* (de Man). *Biological Forum – An International Journal, 15*(4): 926-935.