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Research Article

PRODUCTION OF DECREASED CALCIUM WATERS FOR SHRIMP (*LITOPENAEUS VANNAMEI*) FARMING USING EDTA

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ABSTRACT

Since a decade few shrimp farmers from Tamil Nadu and most of the farmers from Andhra Pradesh of India are utilising bore well waters for shrimp farming. Most of the times these waters are in high in calcium that may lead to mineral deposition on carapace, delayed molting and stunted growth of the shrimp. In Aquaculture its a regular practice to use chelators like EDTA to reduce hardness and calcium levels in water. In the present study an attempt has been made to decrease the calcium levels of bore water using EDTA at different concentrations at different time intervals for different salinities. The results have shown that the EDTA used for the study has been shown to be worth use at 1.5 ppm concentration for the selected salinities. Potential decrease in pH was observed at low salinities with 1.5 and 2ppm EDTA and same is the case with Alkalinities with 2ppm. The calcium and magnesium were found to decrease with 1.5 and 2ppm for 15 to 25 salinities 3hrs after the application and much change was not seen after 24 hrs and same is with the hardness.

INTRODUCTION

India is the second largest aquaculture nation in the world with 10.79 million tons of production falling next to china (FAO, 2016). The vast resources in terms of water bodies and species of fish and shellfish in different agro-ecological regions of the country provide for a wide array of culture systems and practices. The shrimp, *Litopenaeus vannamei* is the major contributor to the overall aquaculture production in the country. Due to its growth rate, short culture period and high export value *Litopenaeus vannamei* expanded rapidly across the country (Padmavathi and Darwin 2017). The successful production of shrimp is often limited by physico-chemical factors of the water as it directly influences the productivity of shrimp farm and the ambient water in which shrimp lives has a cumulative effect on growth performance and survival of shrimp with respect to the production(Gupta *et al*., 2001). Proper water quality management plays a significant role to prevent the stress on shrimp that can accelerate them to various diseases (Tharavathy 2014). Therefore, study of physico-chemical factors is the focal point of much research (Adhikari *et al*., 2017 and Jana *et al*., 2000) to prevent diseases and other changes that cause stress to shrimp.

The calcium and magnesium levels in water are very essential element for *L vannamei*. The magnesium concentration must be at least three times than the calcium concentrations. The calcium and magnesium values should follow the formula that Calcium (Ca^{+2}) in ppm = Salinity x 11.6

Magnesium (Mg^{+2}) in ppm= Salinity x 39.1(CIBA extension, 2016)

Magnesium serves as a cofactor for several enzymes important for the metabolism of proteins, carbohydrates, and lipids (Davis and Gatlin 1996). Magnesium is necessary for forming specific enzyme complexes that necessary for osmotic and ionic regulation in acclimation of shrimp to lower salinity water. Magnesium is also necessary for neuromuscular transmission and skeletal tissue metabolism (Cheng *et al.,* 2006).

Calcium is an important element required for calcification in the hardening cuticle. In addition to this role, calcium helps to maintain physiological homeostasis. Calcium is absorbed from the external environment, recycled from discarded cuticle, and obtained from food items. Calcium absorbed by the gills and gut is transferred by the epidermis to the cuticle, or is stored in the hemolymph or in other tissues such as the hepatopancreas. Crustaceans continually molt their outer shells throughout their lives as they grow. Metabolism, reproduction, and behaviour are directly and indirectly affected by periodic ecdysis (Passano, 1960). Calcium regulation is also affected by the molting cycle. The mechanisms of calcium absorption,

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transportation, and storage vary during the molting cycle. High calcium levels may lead to mineral depositions on carapace of shrimp and sometimes delayed moulting. Since a decade, a few shrimp farmers of Tamil Nadu, India have been using saline borewell water for shrimp culture, and they are able to avoid the outbreak of viral diseases (Gopalakrishnan *et al*., 2008). However when the bore-well water was used, mineral deposition was noticed on the farm implements like aerators, PVC pipes, electric wires, concrete structures (like sluices) and on the shrimps too (Gopalakrishnan *et al*., 2008). The mineral deposition made the shrimp's shells rough (rough shell disease), stunted the growth of the shrimp and caused more mortality resulting in less survival rate (Gopalakrishnan *et al*., 2011). Now- a- days in Andhra Pradesh, it has become a regular habit of utilising bore well waters for shrimp farming as a part of avoiding creek based contaminations where most of the bore waters were rich of calcium concentration than required for shrimp farming (Jaganmohan and Leela Kumari, 2018). In such conditions there is high necessity of decreasing calcium in water using chelators. Currently, the most used chemical to experimentally produce calcium-free water is EDTA (ethylene diamine tetra acetic acid) (Pinheiro et al 2011). EDTA is a synthetic compound that complexes itself with free calcium in water and withdraws it from solution (APHA, 1999). In addition to calcium, EDTA is also capable of chelating with several others elements, including some that may be toxic to animals (Oviedo; Rodríguez, 2003). Currently, EDTA is used routinely in the intensive culture of penaeid shrimp larvae to increase both the percentage of eggs that hatch and the survival of larvae (Robinson *et al*., 2005). Its use, however, must be with caution because EDTA can also be toxic to aquatic animals at higher concentrations (Nowack, 2002). . In the present study we aimed at the production of reduced calcium waters for utilising in *L. vannamei* farming.

MATERIALS AND METHODS

The trials were performed at Matrix Aqua Laboratory, Nellore, Andhra Pradesh, India

Collection of water samples

Water from pond filled with bore well with high calcium was collected from Payanampuram village, Kavali Mandal, SPSR Nellore district, Andhra Pradesh state, India. The salinity of the collected water was measured using refractometer and was adjusted to 30 ppt.

***Water quality analysis*:** The water quality parameters like pH, salinity, carbonates and bicarbonates, Total Alkalinity and Hardness were measured before the addition of EDTA. pH was measured using digital pH meter (Electronics India), and Salinity with refractometer (Erma). Alkalinity and hardness were estimated using (APHA, 1999) standard protocols.

Dilutions

The water collected was further diluted with tap water to the required salinities (5, 10, 15, 20 and 25ppt). For each salinity it is further divided into five equal parts each volume 1 litre and marked as T1, T2, T3, T4 and keeping one as control.

Treatment with EDTA

Commercial EDTA was obtained from Matrix Sea Foods India Limited, Hyderabad and weighed as per the concentrations required (1ppm, 1.5ppm, 2ppm, 2.5ppm) using digital weighing balance and added to T1, T2, T3, T4 of each salinity waters keeping control without addition of EDTA.

Determination of water quality after EDTA treatment

Water quality analysis of treatment samples were performed as per the above protocols at different time intervals viz. 1hr, 3hrs and 24hrs and results were compared for different salinities at different time intervals.

RESULTS

pH

For all EDTA treated samples at different concentrations (1ppm, 1.5ppm, 2ppm, and 2.5ppm) pH was tested for 1hr, 3hr and 24 hrs at for different salinities of 5, 10, 15, 20, 25 and 30. Potential decrease in pH was observed at 5, 10, 15 salinities for 3 hr at 1.5ppm and 2ppm concentration. EDTA seemed to be more effective in decreasing the pH value at 5 ppt (Fig.1).

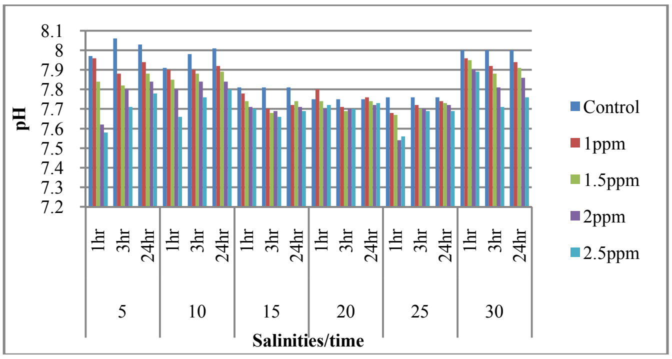

Fig 1 Changes in pH at different concentrations of EDTA at different salinities and time intervals

Total Alkalinity

For all EDTA treated samples at different concentrations (1ppm, 1.5ppm, 2ppm, and 2.5ppm) total alkalinity was tested for 1hr, 3hr and 24 hrs at different salinities of 5, 10, 15, 20, 25 and 30. Maximum decrease in Total alkalinity was observed at 10 & 15 salinity for 3 hr at 2ppm concentration. Not much difference was observed at 25 and 30 ppt salinity (Fig.2.)

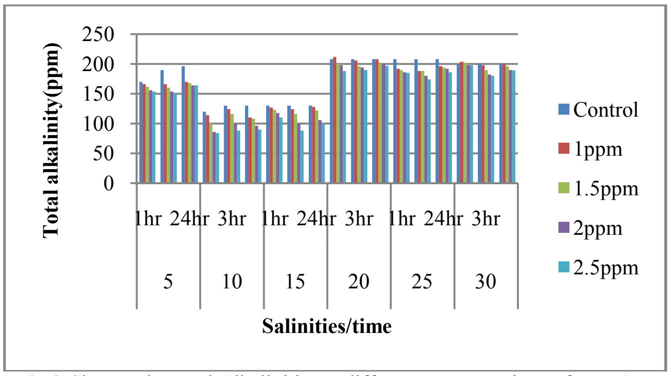

Fig 2 Changes in Total Alkalinities at different concentrations of EDTA at different salinities and time intervals

Calcium (Ca^{+2})

Calcium hardness is the crucial parameter to be tested to know the effect of EDTA. For all EDTA treated samples at different concentrations (1ppm, 1.5ppm, 2ppm, and 2.5ppm) Calcium hardness was tested for 1hr, 3hr and 24 hrs at different salinities of 5, 10, 15, 20, 25 and 30. Maximum decrease in Calcium levels were observed at 15salinity for 3 hr at 1.5ppm and 2ppm concentrations of EDTA. Potential decrease in calcium was also observed at 25 salinity with 1.5ppm EDTA concentration and at 30 salinity with 2ppm EDTA concentration in the first hour of treatment and also with 1ppm concentration for 3hr. There is no significant variation at 5ppm concentration of all the EDTA treated samples when compared to control (Fig.3).

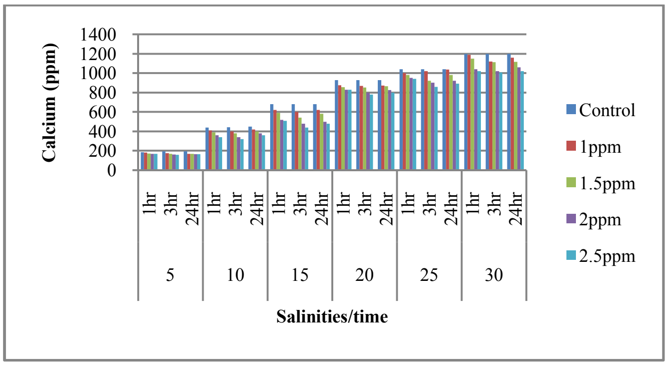

Fig 3 Changes in calcium (Ca^{+2}) levels at different concentrations of EDTA at different salinities and time intervals

Magnesium (Mg^{+2})

With different concentrations (1ppm, 1.5ppm, 2ppm, and 2.5ppm) of EDTA treated samples the magnesium was found to decrease different salinities of 5, 10, 15, 20, 25 and 30. Maximum decrease in magnesium levels were observed at 30 salinity for 3 hr with 1.5ppm EDTA concentration. But there is no significant change in magnesium levels at 15 and 25 salinities in all time periods (Fig.4).

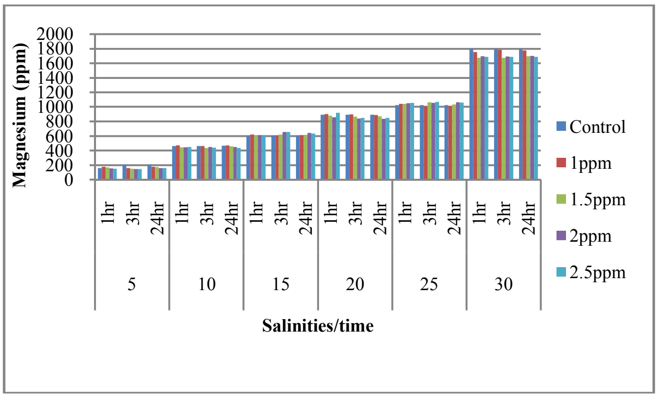

Fig 4 Changes in magnesium (Mg^{+2}) levels at different concentrations of EDTA at different salinities and time intervals

Total Hardness

Decrease in calcium and magnesium levels confers to the decrease in total hardness of the water sample. For all EDTA treated samples at different concentrations (1ppm, 1.5ppm, 2ppm, and 2.5ppm) total hardness measured was found to decrease for all salinities with 1.5ppm EDTA. No significant difference was observed in all salinities (Fig.5).

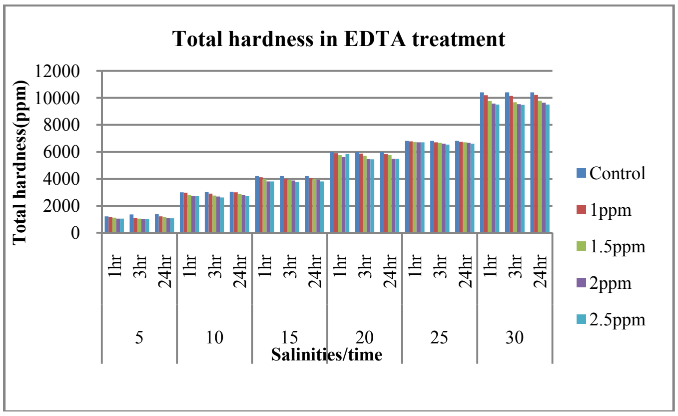

Fig 5 Changes in Total Hardness levels at different concentrations of EDTA at different salinities and time intervals

DISCUSSION

Decrease in pH was observed at first 1hr and 3 hr of 2ppm concentrated EDTA application (Fig.1.). The initial drop in water pH after EDTA application was already expected since EDTA is an acidic compound (Harris, 2007). The pH remained stable at 20salinity in all application rates of different EDTA concentrations. The expressive increase in water pH after 24hr for EDTA application rate of 1ppm and 1.5 ppm can be explained by the reaction between CO_3^{-2} and H_2O. In this case, as the EDTA withdrew Ca^{2+} from solution, CO_3^{-2} became free to react with H_2O and release OH^-, according to the following reaction: $CO_3^{-2}+H_2O \rightarrow HCO_3^- + OH^-$ (BOYD, 1979). The same however did not happen in the EDTA application rates of 2ppm and 2.5ppm because the significantly higher concentrations of H^+ in the water were capable to neutralize the effect of OH^- in lowering the pH of water. It was also observed that sodium salts of amino polycarboxylates, such as the one used in the present work, may have an alkaline reaction with water (Schmidt and Brauch, 2004). As EDTA produces significant effects on water pH, it is necessary to correct the pH of water after the EDTA application with some acidic or alkaline solution, depending on the case.

Water hardness is a measure of the amount of calcium and magnesium salts dissolved in water. Calcium in addition to its structural functions, calcium plays an important role in muscle contraction, blood clot formation, and nerve impulse transmission, the maintenance of cell integrity and acid - base equilibrium, and activation of several important enzymes.

The uptake of calcium occurs through gills, fins and oral epithelia, however gills are considered the most important site for calcium regulation. But increased calcification could present a mechanical (and ecological) problem for shrimp. Increase in mineral to matrix ratio will make biological structures more stiff, but also more brittle. For example carapace primarily serves to protect the internal organs and may fracture more easily during predatory attempts. Like the other bio elements, Ca shows a massive EDTA dose-dependent mobilization (Jastrzebska, *et al.*2009).

The application of EDTA results in the binding of calcium ions with EDTA to form EDTA-Ca, hence the application of EDTA dereased the calcium concentration in water effectively, the more the concentration the more the decrease in calcium. In the present study there is maximum reduction in Calcium levels at 15salinity for 3 hr at 1.5ppm and 2ppm concentrated EDTA.

Potential decrease in calcium hardness was also observed at almost all salinities with increase in concentration of EDTA.

Magnesium is an essential cofactor in many enzymatic reactions in intermediary metabolism. These enzymes include phosphokinases, thiokinases, phosphatases, pyrophosphatases, and amino acyl synthetases. Magnesium plays an important role in the respiratory adaptation of neuromuscular transmission (Houston,*et al*.,1985). When water is treated with EDTA, it can also bind with Magnesium ions to form EDTA-Mg complex. In our study maximum decrease in Magnesium ionic levels was observed in 30 salinity.

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