

Original Research Article

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Role of Minerals Supplementation on Growth and Survival of *Litopenaeus vannamei* in Low Salinity Water

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ABSTRACT

The present study “Effect of Aqueous and dietary minerals supplementation on growth and survival of *Litopenaeus vannamei* in low salinity water” was conducted in the Krishi Vigyan Kendra, Kampasagar, Nalgonda of Professor Jayashankar Telangana state Agriculture University. *L. vannamei* culture in inland low salinity water in currently being practiced various parts of the country. These environments are often deficient in key ions essential for normal physiological function of shrimp, which resulted in reduced growth and mortalities. In the present study it has been evaluated to identify the efficient source mineral supplementation for *L. vannamei* in low salinity waters. Aqueous and dietary mineral supplementations were tested for the efficiency on survival and growth of *L. vannamei* in low salinity water. Experiments were conducted at 3 ppt salinity bore well water for throughout the period of 7 weeks. Dietary mineral supplementation source was proved to be the best option for mineral supply for *L.vannamei* in low salinity water culture. In the dietary mineral supplementation treatments highest growth performance of 3.92g and survival (80%) were recorded for potassium (K⁺10g and Na⁺ 20g per kg diet) supplementation. FCR lowest is recorded in potassium dietary mineral supplementation treatment. Highest growth performance in vannamei was observed for magnesium (Mg²⁺ 80mg l⁻¹) addition to that water among all aqueous mineral supplementation treatment. The highest survival of 70% was recorded for potassium (30mg l⁻¹) treatment among all aqueous mineral supplementation treatment. All the aqueous and dietary mineral supplementation treatments indicated highest growth and survival than control treatment.

Keywords

Litopenaeus vannamei, Shrimp, Dietary mineral, growth

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Introduction

The culture of shrimp and other fish and crustaceans using low salinity water is a trend that continues to grow throughout the world. In 2011, aquaculture accounted for 52.5% of the world’s fish food supply (FAO 2011). Most fish, crustacean and mollusc aquaculture production (61%) occurs in inland waters. In

the same year, brackish water production accounted for 8%. In most locations throughout the world the primary candidate of choice for shrimp culture in low salinity water is the Pacific white shrimp, *Litopenaeus vannamei*, which is native to the Pacific coast from Northern Peru to Mexico. In 2011, *L. vannamei* production worldwide was close to 2.5 million tonnes, which is roughly 71% of

total shrimp and prawn production worldwide (FAO 2011). All indications are that the production of *L. vannamei* will continue to expand, particularly in countries such as China, Vietnam and Thailand. The Pacific white shrimp is a euryhaline species that can tolerate a wide range of salinities 0.5 – 45 g L⁻¹ (Menz and Blake 1980; Bray *et al.*, 1994). There are even some indications that it is capable of growing in waters of less than 0.5 g L⁻¹ (Araneda *et al.*, 2008; Cuvin-Aralar *et al.*, 2009). The remarkable ability of *L. vannamei* to grow in less than ideal environments has made it the species of choice for culturing in low salinity water (Dall *et al.*, 1990, Rothlisberg, 1998 and Alday-Sanz, 2010).

To overcome these disease problems, one proposed solution to whiteleg shrimp production problems is the use of water with salinities lower than sea water. Several researchers (Van Wyk *et al.*, 1999; McGraw *et al.*, 2002; Saoud *et al.*, 2003; Sowers and Tomasso 2006a) have studied the growth of *L. vannamei* in different salinities. In the same way an attempt has been made in zero salinity to study the growth of *L. Vannamei*.

During the last few years, white spot disease (WSD) has spread worldwide and caused large scale mortalities and severe damage to shrimp culture, particularly in Asia leading to massive economic losses. Due to continuous outbreak of WSSV in of *P.monodon* culture leads to shattering of shrimp culture in India. So the farmers are seriously looking for alternative species for culture. At right time the Coastal Aquaculture Authority of India (CAA) introduced a new species (*Litopenaeus vannamei*) in India.

At the same time CAA is very keen in the bio security and approval for culture of *L. vannamei*. The shrimp has been introduced and farmed in Asia since the mid 1990s, with production in Mainland China being

particularly significant. However, beginning in 1996, *L. vannamei* was introduced into Asia on a commercial scale. Total production of *L. vannamei* in Asia was approximately 3, 16,000 mt in 2002.

It is now evident that *L. vannamei* is farmed and established in several countries in East, Southeast and South Asia and is playing a major significant role in shrimp aquaculture production. There is very limited research works were done on the culture and growth performance of *L. vannamei* with different stocking densities in brackish water ponds in India. Owing to its ability to grow and survive in low salinity environments the Pacific white shrimp, (*Litopenaeus vannamei*, Boone) has become the candidate of choice for low salinity culture.

Inland production of *L. vannamei* in low-salinity water is a growing industry in several regions of the world. Depending on their source, inland waters available for shrimp culture are usually of different salinities and possess different ion compositions (Boyd and Thunjai, 2003). Inland shrimp culture has been practiced for several years with tiger shrimp in Thailand (Flaherty and Vandergeest, 1998), and white shrimp has been cultivated inland in several regions of the United States (Davis *et al.*, 2002). Factors such as absence of white spot syndrome virus (WSSV) in water sources (Sanchez-Barajas *et al.*, 2009), adequate environmental temperatures year around, low equipment corrosion and proximity to large markets have permitted the establishment of several farms totalling 350 ha, which produced in 2007 over 1500 metric tons (Industria Acuicola, 2008) have permitted for the expansion of culture in inland low salinity waters.

A dietary source of 23 minerals has been demonstrated as essential in 1 or more animal species. These elements are divided into two

groups: the 7 macrominerals (calcium, chlorine, magnesium, phosphorus, potassium, sodium, and sulfur) and 16 trace minerals (aluminium, arsenic, cobalt, chromium, copper, fluorine, iodine, iron, manganese, molybdenum, nickel, selenium, silicon, tin, vanadium, zinc). Thirteen minerals are required in the diet of most terrestrial animals and may be essential for aquatic animals. Among these minerals, eight are cations: calcium(Ca^{2+}), copper(Cu^{2+}), iron(Fe^{2+}), magnesium(Mg^{2+}), manganese(Mn^{2+}), potassium(K^+), sodium(Na^+), and zinc(Zn^{2+}); and five anions or are usually found in anionic groupings: chloride(Cl^-), iodine(I^-), molybdenum(Mo^{2-}), phosphate(PO_4^{3-}) and selenite(SeO_3^{2-}) (Scott *et al.*, 1982).

With the exception of osmoregulation, the maintenance of osmotic balance between body fluids and the water in which the animal lives, the biochemical functions of minerals in aquatic species appear to be similar to those in terrestrial animals (Lovell, 1989). Fresh water species lose ions to the hypotonic environment and therefore suffer from dehydration, whereas the reverse is true for marine species. Unlike terrestrial animals, which are primarily limited to a dietary source of minerals, aquatic animals may be able to utilize, to some extent, minerals dissolved in the water to meet physiological requirements. Calcium, Copper, Iron, Magnesium, Potassium, Sodium, Selenium, and Zinc are generally derived from the water to satisfy part of the physiological requirements of fish (National Research Council, 1993).

Since aquatic animals can obtain minerals from both ambient water and feed, dietary supplements of selected minerals could facilitate better survival and growth of shrimp held in low salinity conditions. If one looks at the mineral profile of most low salinity well waters as compared to the profiles of low salinity water of oceanic origin you find that

the levels of potassium and magnesium are much lower in the low salinity well water source. A marine species reared in seawater do not require dietary sources of magnesium and potassium, whereas freshwater species reared in freshwater do. Consequently, these minerals may be low in marine shrimp feeds that are fed to marine shrimp reared in low salinity water.

Recommendations and research into the influence of minerals on growth and survival in low salinity water is warranted. The ionic composition of saline water appears to be more important than salinity with regards to its effect on shrimp survival and growth. This is probably due to the fact that most of these waters contain adequate levels of sodium and chloride to meet the shrimp's physiological requirement. However, other ions are not at sufficient levels in the water or possibly the diet to meet physiological requirements. Quite often inland shrimp farmers complain of a slow die-off of shrimp. They report that shrimp are easily stressed by handling, temperature and low dissolved oxygen levels of the water. The farmers often observe lethargic shrimp along the sides of nursery tanks and ponds, or stressed shrimp even after gentle handling. Stress is often characterized by a whitening of the tail, cramping and possibly death. A probable reason for this response would be ionic imbalances and nutrient reserves caused by this unique environment.

The objectives of present study are:

The effect of identified minerals like (Na, K and Mg) supplements through aqueous and dietary source on growth and survival of *L. vannamei*.

Among the both aqueous and dietary minerals supplementation which gives better growth and survival of *L. vannamei* in low salinity water.

Materials and Methods

Site of the experiment

The experiment was conducted in Krishi Vigyan Kendra, Kampanagar, Nalgonda of Professor Jayashankar Telangana state Agriculture University for a period of 7 weeks.

Experimental animals and their acclimatization

Litopenaeus vannamei (1000 numbers) were obtained from CP Hatchery, Nellore, who has been authorized by Coastal Aquaculture Authority (CAA), Chennai to produce seed. Post larvae (PL10) transported by road in plastic bags containing 15 ppt saline water. PL transferred to the same salinity water in the wet lab. Acclimatization was carried out over 8 days. During this time salinity was lowered from 15 ppt to 3ppt bore well water at an average rate of 4ppt day⁻¹ (M. Araneda *et al.*, 2008). During this period the seed were fed with control diet. Shrimp seed were packed in double plastic bags filled with oxygen and water in the ratio of 3:1 in each bag and the density of shrimp was 300/bag. The number of shrimp seed to be packed in oxygen inflated polythene bags was calculated as per the following formula (Jameson *et al.*, 1995).

$$N = (DO - 2) \times V / CH$$

Where:

DO: Dissolved oxygen content of water (mg/l)

V: Volume of water used for transport (Lt)

C: Rate of oxygen consumption of shrimp (ml/kg of shrimp)

H: Duration of transport (Hours)

Experimental design

The aquarium tanks used for experiments were of size 60x30x30 cm (Plate 1). Twenty one

aquariums were stalked on iron racks. Aquariums were located in a secured place where there is no direct sunlight and covered all the sides with black paper to avoid algal growth in the tank. Water in the aquariums was aerated by using air stones connected to the air compressor. Filters are used for filtering the aquarium water. The underground water was taken into a tank and allowed to aerate for 48 hours and was used for filling the aquaria. Salinity was checked before taken the water into aquarium. The water is allowed to filter for 24 hours before introducing the shrimps into the aquaria.

Ten numbers of Shrimps with initial average weights of 0.15 – 0.18gm were introduced in to each aquarium and triplicates were maintained for each treatment (Dietary supplementation of (plate 2) Na-10g, Na-20g, K-5g, K-10g, Mg-150mg, Mg-300mg and Aqueous supplementation of K-20mg, K-30mg, Mg-40mg, Mg-80mg) includes control. Regular water exchange of 25% was done every day. Left over feed, excreta and other debris was siphoned off from the bottom of the tank without disturbing the shrimps.

Experimental feed preparation and Feeding

In the experiment, formulated feed with the crude protein (35%) were used for feeding. Fishmeal, soybean meal, groundnut oil cake, maize and deoiled ricebran were the ingredients used for control feed. Experimental diets were prepared with same ingredients as used in control diet. In addition to that experimental diet contained following mineral 5g potassium (K⁺). Each diet was prepared separately by adding 10g potassium, 10g sodium, 20g sodium, 150mg magnesium, 300mg magnesium. 1% of vitamin mixture added to experimental diets. All the ingredients that are Soybean meal, deoiled rice bran, maize, ground nut oil cake, vitamins used in feeds were obtained from local

markets. Ingredients used in the feed and all the experimental diets were estimated for proximate composition (AOAC, 1995).

Each ingredient was procured in required quantity and ground into powder and sieved. All the ingredients were then mixed in required proportion and water was added at the rate of 30 ml per every 100g of feed and dough was prepared. Maida (1%) was used as a binding agent in the feed. The dough was cooked for 20 minutes in pressure cooker and then cooled. 1% Vitamin mixture was added. The homogenous dough was pressed through a hand pelletizer (La Monferrina s.r.l, Italy) with a sieve of 1 mm diameter. The feed was dried in shade and then in hot air oven at 80-90°C to reduce the moisture content to 10% and stored properly in dry and air tight bottles and kept in dark cool place.

Proximate composition

Proximate analysis of the feed was estimated by the method of AOAC, 1995.

Results and Discussion

Growth

Growth of *L. vannamei* fed with dietary minerals supplementation

Weight of shrimp in grams and weight increment data observed weekly for different treatments. Observations on the growth during the first week (7th day) revealed that weight increment varied between 0.41±0.04 and 0.50±0.10gms for treatment control and Na-10g respectively. Highest and lowest average weight values were observed in the treatments K-10g (0.68±0.02) and control (0.57±0.12). On the 14th day highest increment of 0.54±0.12gms and lowest increment of 0.41±0.11gms were recorded for the k-10g and control respectively. Highest average

weight values (1.14±0.07) and lowest average weight values (0.98±0.04) were recorded for Na-20g and control respectively during the second sample (14th day). Similar trend continued during the 21st day also. The highest and lowest weight growth of increment observed were 0.51±0.11 and 0.40±0.10gms for Na-20g and K-10g respectively, while the highest and lowest average weight values observed were (1.65±0.09 and 1.43±0.11) for Na-20g and control respectively. Treatments Na-10g and K-10g stood in second and third positions with growth in weight of 1.63±0.12 and 1.62±0.05 respectively. During the 28th day, the highest and lowest weight increments observed were 0.57±0.07 and 0.39±0.05gms for K-10g and control respectively. The highest and lowest average weight values observed were (2.19±0.04 and 1.82±0.07) for K-10g and control respectively. Treatments Na-20g and Mg-300mg stood in second and third positions with weight growth of 2.18±0.07 and 2.16±0.07gms respectively. Similar trend continued during the 35th day of the experiment also. Highest and lowest weight growth increments observed were 0.54±0.04 and 0.46±0.07gms for K-10g and Mg-150mg respectively. The highest and lowest average weight values observed were (2.73±0.12 and 2.30±0.05) for K-10g and control respectively. Mg-300mg and Na-10g stood in second and third positions with growth weight gain of 2.65±0.05 and 2.63±0.14gms respectively. On the 42nd day highest increment of 0.59±0.09gms and lowest increment of 0.36±0.14gms were recorded for the K-5g and control respectively. Highest average weight values 3.21±0.10 and lowest average weight values 2.66±0.03 were recorded for K-5g and control respectively. On the 49th day highest increment of 0.72±0.10gms and lowest increment of 0.42±0.11gms were recorded for the K-10g and control respectively. Highest average weight values 3.92±0.06 and lowest average weight values 3.08±0.07 were recorded for K-

10g and control respectively. Also highest average weight values of 3.92 ± 0.06 from K-10g and lowest average weight values of 3.08 ± 0.07 from control were observed at the end of the experiment. An overall study indicated that the K-5g recorded total weight increment of 3.87 ± 0.07 g in the 49 days experimental period. This was followed by the Na-20g (3.71 ± 0.08), Na-10g (3.70 ± 0.04 gm) and Mg-300mg (3.69 ± 0.08 gm), they stood in second, third and fourth positions respectively.

The growth data was subjected to analysis of variance (ANOVA) at 5% level of significance and the observations were presented. The statistical analysis has shown that F- value is found to be significant among treatments. Since F- value is found to be significant, the pair wise comparison of any two Treatments could be done by computing RBD two way classification. The Treatment K-10g is found to be significantly superior when compare to other Treatments. Treatment K-10g has shown significantly different from all other Treatments. The second and third positions were occupied by Na-20g and K-5g respectively. There was a significant difference between the culture periods also.

Growth of *L. vannamei* supplied with aqueous minerals

Weight of shrimp in grams and weight increment data observed weekly for different treatments were studied. Observations on the growth during the first week (7th day) revealed that weight increment varied between 0.41 ± 0.04 and 0.48 ± 0.07 gms for treatment control and K-20mg respectively. Highest and lowest average weight values were observed in the treatments Mg-80mg (0.64 ± 0.05) and control (0.57 ± 0.12). On the 14th day highest increment of 0.49 ± 0.10 gms and lowest increment of 0.41 ± 0.11 gms were recorded for the Mg-80mg and control respectively. Highest average weight values (1.13 ± 0.14)

and lowest average weight values (0.98 ± 0.04) were recorded for Mg-80mg and control respectively during the second sample (14th day). Similar trend continued during the 21st day also. The highest and lowest weight growth of increment observed were 0.47 ± 0.09 and 0.37 ± 0.14 gms for Mg-80mg and K-30mg respectively, while the highest and lowest average weight values observed were (1.60 ± 0.02 and 1.43 ± 0.04) for Mg-80mg and K-30mg respectively. Treatments K-20mg and Mg-40mg stood in second and third positions with growth in weight of 1.56 ± 0.04 and 1.45 ± 0.02 respectively. During the 28th day, the highest and lowest weight increments observed were 0.45 ± 0.12 and 0.39 ± 0.02 gms for Mg-80mg and K-30mg respectively. The highest and lowest average weight values observed were (2.09 ± 0.07 and 1.82 ± 0.01) for K-20mg and K-30mg respectively. Treatments Mg-80mg and Mg-40mg stood in second and third positions with weight growth of 2.05 ± 0.11 and 1.86 ± 0.07 gms respectively. Similar trend continued during the 35th day of the experiment also. Highest and lowest weight growth increments observed were 0.53 ± 0.07 and 0.44 ± 0.05 gms for K-30mg and Mg-40mg respectively. The highest and lowest average weight values observed were (2.55 ± 0.11 and 2.30 ± 0.05) for K-20mg and control respectively. Mg-80mg and K-30mg stood in second and third positions with growth weight gain of 2.51 ± 0.12 and 2.35 ± 0.05 gms respectively. On the 42nd day highest increment of 0.60 ± 0.05 gms and lowest increment of 0.36 ± 0.14 gms were recorded for the Mg-80mg and control respectively. Highest average weight values 3.11 ± 0.04 and lowest average weight values 2.66 ± 0.03 were recorded for Mg-80mg and control respectively. On the 49th day highest increment of 0.56 ± 0.12 gms and lowest increment of 0.42 ± 0.11 gms were recorded for the K-20mg and control respectively. Highest average weight values 3.65 ± 0.07 and lowest average weight values 3.08 ± 0.07 were

recorded for Mg-80mg and control respectively. Also highest average weight values of 3.65 ± 0.07 from Mg-80mg and lowest average weight values of 3.08 ± 0.07 from control were observed at the end of the experiment. An overall study indicated that the K-20mg recorded total weight increment of 3.64 ± 0.05 g in the 49 days experimental period. This was followed by the Mg-40mg (3.30 ± 0.05), K-30mg (3.28 ± 0.07 gm) they stood in second and third positions respectively.

The growth data was subjected to analysis of variance (ANOVA) at 5% level of significance and the observations were studied. The statistical analysis has shown that F- value is found to be significant among treatments. Since F- value is found to be significant, the pair wise comparison of any two Treatments could be done by computing RBD two way classification. The Treatment Mg-80mg is found to be significantly superior when compare to other Treatments. Treatment Mg-80mg has shown significantly different from all other Treatments. The second and third positions were occupied by K-20mg and Mg-40mg respectively. There was a significant difference between the culture periods also.

Survival rate

Survival of *L. vannamei* fed with dietary minerals supplementation

Survival percentages of *L. vannamei* shrimp in various experimental treatments are presented. The survival percentage throughout the period of experiment was lowest for the control, Mg-150mg, Mg-300mg, k-5g, Na-10g, Na-20g, K-10g. By the final sampling (49th day) the survival percentage was (highest) 80.0 - and (lowest) 50.0.

The survival data was subjected to analysis of variance (ANOVA) presented. Statistical

analysis has shown that F- value is found to be significant among treatments. Since F- value is found to be significant, the pair- wise comparison of any two treatments could be done by computing RBD two way classifications. The treatment K-10g had shown highest survival rate when compared to the other treatments. The subsequent positions were occupied by Treatments Na-20g, K-5g, Mg-300mg, Na-10g, Mg-150mg followed by control. Treatment K-10g has shown significant difference from all other treatments. There was significant difference in between experimental period also.

Survival of *L. vannamei* supplied with aqueous minerals

Survival percentages of *L. vannamei* shrimp in various experimental treatments were presented. The survival percentage throughout the period of experiment was lowest for the control, K-20mg, Mg-40mg, Mg-80mg and K-30mg. By the final sampling (49th day) the survival percentage was (highest) 70.0 - and (lowest) 50.0.

The survival data was subjected to analysis of variance (ANOVA) presented. Statistical analysis has shown that F- value is found to be significant among treatments. Since F- value is found to be significant, the pair- wise comparison of any two treatments could be done by computing RBD two way classification. The treatment K-30mg had shown highest survival rate when compared to the other treatments. The subsequent positions were occupied by Treatments K-30mg, Mg-80mg, Mg-40mg, K-20mg followed by control. Treatment K-30mg has shown significant difference from all other treatments. There was significant difference in between experimental period also.

Specific growth rates

Specific growth Rates of *L. vannamei* fed with dietary minerals

Supplementation

Specific growth rates for *L. vannamei* treated with different diets were calculated and studied. The specific growth rates by end of the experimental period (49 days) were calculated for all treatments.

Control group has the lowest Specific Growth Rate of 6.03%. The highest value was in Mg-150mg with 6.50%. The treatments that stood second and third positions were Na-10g (6.41%) and Mg-300mg (6.40%). These were followed by K-10g (6.28%), K-5g (6.26%) and Na-20g (6.17%) respectively.

Specific growth rates of *L. vannamei* supplied with aqueous minerals supplementation

Specific growth rates for *L. vannamei* (whiteleg shrimp) treated with different diets were calculated and studied. The specific growth rates by end of the experimental period (49 days) were calculated for all treatments.

Control group has the lowest Specific Growth Rate of 6.03%. The highest value was in K-20mg with 6.50%. The treatments that stood second and third positions were Mg-80mg (6.38%) and Mg-40mg (6.17%). These were followed by, K-30mg (6.16%).

Feed conversion ratio

Feed conversion ratio of *L. vannamei* fed with dietary minerals supplementation

The Feed Conversion Ratio in different experiments of *L. vannamei* groups were calculated and presented. The range for Feed Conversion Ratio observed during the period of experiment was 0.20 (Mg-150mg) – 3.68(control).

During the first sampling (7thday) Feed Conversion Ratio ranged between 0.20 and 0.30 and the highest during this period was recorded for k-5g,Na-20g and the lowest was for Mg-150mg.

Sampling on the 14th day shown the highest value 1.00 for Na-20g and the lowest 0.82 for K-5g. The highest value of 1.92 was observed for K-10g on 21th day while the lower of 1.28 was recorded for Mg-150mg.

The sampling on 28th day recorded control with highest Feed Conversion Ratio value 2.29 as same reading and lowest value of 1.73 for treatment of Na-20g.

The highest value of 2.41 was observed for control on 35th day while the lowest of 2.27 was recorded for K-10g. Sampling on 42nd day recorded highest value of 3.50 for control and lowest value of 2.47 for K-5g. The last sampling on 49th day recorded control with highest Feed Conversion Ratio value 3.68 and lowest value of 2.54 for treatment of K-5g.

The Feed Conversion Ratio was subjected to analysis of variance (ANOVA) and presented. Statistical analysis has shown that F- value is found to be significant among treatments. Since F- value is found to be significant, the pair- wise comparison of any two treatments could be done by computing RBD two way classification.

The control was found to be significantly superior when compared to the other Treatments. The K-10g and Na-10g occupied second and third positions. There was a significant difference between the experimental periods also.

Feed conversion ratio of *L. vannamei* with aqueous minerals

Supplementation

The Feed Conversion Ratio in different experiments of *L. vannamei* groups were calculated and presented.

The range for Feed Conversion Ratio observed during the period of experiment was 0.21 (K-20mg) – 3.68 (control).

During the first sampling (7th day) Feed Conversion Ratio ranged between 0.21 and 0.27 and the highest during this period was recorded for control and the lowest was for K-20mg. The 14th day sampling shown the highest value 0.98 for Mg-40mg and the lowest 0.90 for K-20mg. The highest value of

1.74 was observed for K-30mg on 21th day while the lowest of 1.51 was recorded for Mg-80mg. The sampling on 28th day recorded control with highest Feed Conversion Ratio value 2.29 as same reading and lowest value of 1.85 for treatment of Ka-20mg. The highest value of 2.91 was observed for K-30mg on 35th day while the lowest of 2.41 was recorded for control. The range on 42nd day recorded highest value of 3.50 for control and lowest value of 2.50 for Mg-80mg. The sampling on 49th day recorded control with highest Feed Conversion Ratio value 3.68 and lowest value of 2.88 for treatment of K-20mg.

The Feed Conversion Ratio was subjected to analysis of variance (ANOVA) and presented in table 1. Treatments found to be non significant (Plate 3 and 4).

Table.1 Proximate compositions of the various ingredients used in formulate feeds

S.No	Ingredients	Moisture %	Crude protein%	Crude Fat%	Crude Fibre%	Ash %
1	Fish Meal	8.60	58.10	10.00	0.30	19.20
2	Soya bean meal	10.80	38.00	5.90	1.30	5.30
3	Groundnut oil cake	6.00	37.70	13.80	13.20	7.30
4	Deoiled rice bran	7.20	12.10	1.30	15.20	23.80
5	Maize	15.90	8.00	4.80	1.10	1.40

Plate.1 Experimental set up in the KVK



Plate.1.1 Experimental setup for dietary minerals supplementation



Plate.1.2 Experimental setup for aqueous minerals supplementation



Plate.1.3 Experimental animals fed with control diet



Plate.2 Practical diets used in the experiment



Plate.3 *L. vannamei* length measurement



Plate.4 *L. vannamei* weight measurement

As the production of shrimp in inland low salinity waters continues to expand, so does the need for cost effective methods for increasing the availability of essential ions to the organisms in order to ensure proper growth and survival. Traditional practices, such as the application agricultural fertilisers (k-mag and murate of potash), commercial mineral mixtures application directly to the water without knowing the demand of shrimp, have been proven effective at improving growth and survival (Mc Nevin *et al.*, 2004). However, the use of these minerals needs to be optimised based on demand of the aquatic organism rather than dumping them in to the pond. It may either allow reduction in the level of supplementation of these minerals and also the risk of mortality of the animals. Experiments in the present study were concluded at a salinity of 3 ppt, which is

comparable with the salinity utilised by commercial shrimp farms where the bore wells are the basic source of water. Maintenance of sodium, potassium and magnesium is necessary for proper physiological functioning of body, osmoregulation, building of body and also as activities for many enzymes which play role in carbohydrate metabolism and protein synthesis (Davis *et al.*, 2005).

Growth of *L. vannamei* in aqueous and dietary minerals supplementation

Dietary supplementation of NaCl has the potential to provide benefits for euryhaline species. In the present study growth was enhanced (3.71g) with the increase of sodium concentration (Na^+ 20g kg^+) in the diet. In two separate studies with juvenile red drum

(*Sciaenops ocellatus*) reared in freshwater, growth and feed efficiency were improved when fish were fed a diet supplemented with sodium (Holsapple, 1990; Gatlin *et al.*, 1992). Similar feed efficiency was observed at sodium 10g and 20g level in the diet. The results demonstrated that, upto 10g kg⁻¹ supplementary sodium in experimental diet improved the specific growth rate as reported in pacific whiteleg shrimp in USA (Roy *et al.*, 2007b). Potassium plays an important role in the membrane potential of aquatic animals. The present trail showed that there is positive correlation between potassium dietary supplementation and growth enhancement. In this trail shrimp offered with diet contain 10g kg⁻¹ K⁺ yielded significantly (p<0.05) greater weight gain (3.92g) and specific growth rate (6.28%) than the shrimp fed with control diet.

Shiau and Hsieh (2001) were reported that increase of K⁺ in diet increased growth in *P. monodon*. Gong *et al.*, (2004) demonstrated the impact of K⁺ by conducting the trail with and without mineral supplementation in *L. vannamei*. Similar trend in growth enhancement with dietary mineral supplementation was observed by many earlier works in *L. vannamei* (Davis *et al.*, 2005; Muylder *et al.*, 2006 and Roy *et al.*, 2007b). In a field trail supplementation source of chelated K⁺ improved growth in *L. vannamei* (Roy *et al.*, 2007b). However, feed efficiency and feed conversion ratio(FCR)reduced with the increase of K⁺ supplementation from 5g k⁺ kg⁻¹ to 10g k⁺ kg⁻¹. It may be due to higher k⁺ supplementation levels with the increase of osmolality and respiration rates of animals and stress condition FCR reduced compared to earlier trails.

Magnesium is the major constituent of bones and skeletal parts of the animals (Davis *et al.*, 2005). In the current study showed that significant (p<0.05) increase in weight gain of

(3.69g) *L. vannamei* with magnesium supplementation. Magnesium supplement at 300mg kg⁻¹ in practical diet showed better growth than control diet. Similar observation was made by Cheng *et al.*, (2005) in *L. vannamei*. These authors were reported that a dietary Mg⁺² 2.60 – 3.46g kg⁻¹ recommended for optimal growth of *L. vannamei* reared in low salinity water. However, Roy *et al.*, (2007b) observed that there was no significant improvement in growth with magnesium supplementation in practical diet. On the present study higher feed efficiency, lower FCR and higher specific growth rate observed at Mg⁺² 150mg kg⁻¹ supplementation level in the practical diet to that of control diet. Growth of the shrimp was improved when the diet were supplemented with 0.3% magnesium (Kanazawa *et al.*, 1984). Deletion of magnesium from mineral supplemental diet results in reduced tissue mineralisation in *P. vannamei* (Davis *et al.*, 1992).

Aquatic organisms collect most of their required mineral content from the surrounding water. In present study results indicated that the significant(p<0.05) increase in growth, lower FCR and higher specific growth rate at aqueous potassium supplementation 20mg l⁻¹ than to that of control diet. Individual weight gain and specific growth rate and percent weight gain were increased with increasing potassium concentration in aqueous source utilized in vannamei culture in low salinity waters (Roy *et al.*, 2007a). However, in the present study weight gain, specific growth rate and feed efficiency were not increased with the increase of concentration of potassium supplementation from 20mg l⁻¹ to 30mg l⁻¹. Pragnell and Fotedar (2005) found that *Penaeus latissulcatus* reared in low salinity well water with 100% and 80% potassium concentration as compared to sea water resulted in slower growth. Even though potassium concentration increased weight

gain was not observed, it may be due to at higher aqueous potassium concentration in shrimp tissue water decreases and the concentration of free amino acids in the tissue increases. With the progression of above process animal undergo stress it might have resulted in weight loss.

Addition of magnesium to the water from 40mg l⁻¹ to 80mg l⁻¹ increased growth and specific growth rate. Roy *et al.*, (2007a) also noticed similar growth increase in *L. vannamei* low salinity culture with magnesium addition from 10mg l⁻¹ to 160mg l⁻¹. Ahmad Ali (1999) was noticed suppressed growth with the magnesium addition in diet in *Penaeus indicus*. He was opined that the magnesium requirement might be satisfied through absorption from the water. However, feed efficiency did not show significant difference with the magnesium addition to the water.

Survival of *L. vannamei* in aqueous and dietary minerals supplementation

Minerals play a significant role in the survival of pacific white shrimp in inland low salinity water culture. In the present study sodium dietary supplement trails result indicated that increase of survival (80%) with the increase of sodium supplementation from 10g kg⁻¹ diet to 20g kg⁻¹ diet. Roy *et al.*, (2007a) were observed that *L. vannamei* survival increased to 92% from 81%.with an increase of sodium supplementation 20g kg⁻¹ diet to that of control diet. Pequeux (1995) reported that sodium and chloride ions plays significant role in osmoregulation of shrimp. These two ions were essential for the survival of shrimp in low salinity waters. In the present study shrimp offered the diet containing 10g K⁺ kg⁻¹ and 20g Na⁺ kg⁻¹ yielded significantly(p<0.05) more survival than shrimp fed control diet. Our results are supported by Shiau and Hsieh (2001) in *Penaeus monodon*, Pragnell and Fotedar

(2005) in *Penaeus latisulcatus* and Roy *et al.*, (2007b) in *L. vannamei*.

Survival of *L. vannamei* was increased in the diet with magnesium supplementation of 300mg kg⁻¹ to that of control diet. Roy *et al.*, (2007b) were noticed similar increase in survival with the magnesium supplementation by using coating agents. However, Roy *et al.*, (2007b) were observed contrasting results in the other trail without coating agent used in magnesium supplementation diet.

Ahamad Ali, (1999) reported that there was no significance affect on the survival of *P. indicus* with the supplementation of magnesium in the diet. A number of studies were documented the correlation between potassium concentration and the survival of shrimp.(Boyd *et al.*, 2002; Davis *et al.*, 2002; Saoud *et al.*, 2003).

The results of the present study showed that aqueous potassium supplementation is necessary for the survival of vannamei in low salinity water culture. The shrimps have shown higher survival at the addition of potassium 30mg l⁻¹ to the water. Roy *et al.*, (2007a) were observed similar increase in survival of vannamei with the increase of K⁺ in the water. Zhu *et al.*, (2004) were observed improper Na/K ratio in low salinity water made significant impact on survival of *L. vannamei*. Pragnell and Foteder, (2005) were reported that the potassium deficiency in low salinity water culture reduce the *P. latisulcatus* survival.

Results in the present study indicated that addition of magnesium as aqueous source to the vannamei culture in low salinity water enhanced the survival of shrimp. In the present study results showed increasing trend in survival with the increase of magnesium addition to the water. Our observation in the present experiment are also in agreement with previous studies evaluating the impact of magnesium and other minerals

supplementation on survival of *L. vannamei* in low salinity waters (Saoud *et al.*, 2003; Davis *et al.*, 2005 and Roy *et al.*, 2007a). However, Roy *et al.*, (2007a) observed increase of survival in vannamei in low salinity water upto 40mg l⁻¹ of magnesium supplementation to the water, further addition of magnesium resulted in decrease of survival.

In conclusion it can be concluded that dietary minerals supplementation of identified minerals performed better than aqueous minerals supplementation for the enhancement of growth and survival of vannamei in low salinity water.

Further research on role of these minerals in osmoregulation of shrimp may help to answer some of the questions associated with availability in growth and sudden mortality of post larvae in vannamei culture in low salinity water.

Summary

The present experiment was conducted in the wet lab of Department of Aquaculture, College of Fishery Science, Muthukur, SPSR Nellore (District) to study the “Effect of aqueous and dietary minerals supplementation on growth and survival of *L. vannamei* in low salinity water”. Post larvae (PL10) of *L. vannamei* were brought to the wet laboratory from CP hatchery. Post larvae transported by road in plastic bags containing 15 ppt saline water. PL transferred to the same salinity water in the wet lab. Acclimatization was carried out over 8 days. During this time salinity was lowered from 15 ppt to 3ppt bore well water at an average rate of 4ppt day⁻¹ (M. Araneda *et al.*, 2008). After acclimatization to 3ppt, they were transferred to experimental trail and continued mineral supplementation trails.

➤ In the aqueous source mineral

supplementation potassium was added to the water at 20mg l⁻¹ and 30mg l⁻¹. Magnesium was also evaluated as mineral through aqueous source as 40mg l⁻¹ and 80mg l⁻¹ concentration. Dietary supplementations of minerals were assessed with the addition of minerals sodium, potassium and magnesium at different concentration in vannamei culture in low salinity water. In control treatment shrimp fed with control diet. In case of supplementation of dietary minerals in practical diets, required mineral concentration was added to the control diet during the feed preparation. Control treatment shrimp were fed with control diet and compared the aqueous and dietary mineral supplementation for growth and survival of *L. vannamei* in low salinity water.

- The sampling was done weekly. The duration of experiment was 7 weeks. Triplicates were maintained for all the treatments and the control. The results obtained were subjected to statistical analysis.
- The results obtained in the present study on growth, survival, feed conversion ratio and specific growth rate of *L. vannamei* are summarized.
- Important water quality parameters such as dissolved oxygen, temperature, pH, total alkalinity and total hardness were analysed.
- The water quality parameters were recorded in the following order, dissolved oxygen varied between 5.45 – 7.48 ppm, temperature range from 29.0-30.0^oc, pH values ranged between 7.8 - 8.5 and total alkalinity recorded in the range of 240-280 mg l⁻¹. The water quality parameters were observed similar for all the

treatments and control tanks throughout the experimental period.

- Weekly sampling for 7 weeks was done to study growth, survival, FCR, and SGR.
- In the dietary minerals supplementation treatments higher growth performance of 3.92g was recorded, potassium dietary supplementation treatment (K^+ - 10g kg^{-1} diet) and lowest recorded for the control diet.
- All the dietary mineral supplementation treatments were recorded higher growth than to that of control (3.08g).
- The analysis of variance for growth performance showed significantly difference among the treatments.
- Shrimp fed on control diet had showed highest FCR, while potassium dietary supplementation (K^+ - 5g kg^{-1} diet) has shown the least. All the dietary supplementation treatments showed better feed utilization efficiency than control.
- The analysis of variance for FCR has shown significant difference for all the dietary mineral supplementation treatments.
- In the dietary minerals supplementation treatment highest SGR (6.50%) was observed in magnesium dietary supplementation treatment (Mg^{+2} - 150mg kg^{-1} diet) and lowest was recorded for the control.
- The highest survival of 80% and lowest of 50% recorded for shrimps fed on experimental diets with potassium dietary supplementation (K^+ - 10 g kg^{-1} and Na^+ 20 g kg^{-1} diet) and control respectively all the dietary mineral supplementation treatments showed better survival than the control.
- In the aqueous mineral supplementation treatments highest growth performance 3.65g was recorded for magnesium treatment (Mg^{+2} - 80mg l^{-1}) addition to the water and lowest in control.
- All the aqueous mineral supplementation treatments showed better growth performance than to that of control.
- Analysis of variance for growth performance showed significant difference among the treatments.
- FCR observed highest in the potassium addition to the water (K^+ - 20mg l^{-1}) in aqueous mineral supplementation treatments and lowest recorded in control.
- In the aqueous mineral supplementation treatments highest SGR (6.50%) was observed in potassium supplementation (K^+ - 20mg l^{-1}) and lowest recorded for the control.
- The highest survival of 70% and lowest of 50% was recorded in potassium (K^+ - 30mg l^{-1}) addition to the water treatment and control respectively. All the aqueous mineral supplementation treatments demonstrated better survival than the control.
- Analysis variance for the survival showed significant difference among all treatments.
- It can be concluded that dietary mineral supplementation of minerals performed better than aqueous mineral supplementation for the enhancement of growth and survival of vannamei in low salinity water culture.

- Potassium inclusion at (K^+ - 10g kg^{-1} diet) is identified as optimal level of inclusion for vannamei culture in low salinity water.

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