Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(4): 2410-2414

Received: 18-04-2020
Accepted: 22-05-2020

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Investigation of the effect of dietary mineral supplementation on growth of pacific white leg shrimp, *Litopenaeus vannamei* at low salinity water

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Abstract
The importance of the present study, to evaluate to identification of the efficient mineral diet plays the role on growth of *L. vannamei* in low salinity. The shrimps were exposed to different concentration of minerals supplemented trough diet. The experiment was conducted in wet laboratory of College of Fisheries Science for a period of 28 days with five treatments and each treatment triplicate in 0.6x0.3x0.3 m size plastic tubs. The *Litopenaeus vannamei* were fed with four diets containing Ca 0.2% (D_{Ca}), Mg 0.02% (D_{Mg}), K 1% (D_K) and Na 1% (D_{Na}) individually and control diet without addition of extra minerals. The highest average body weight gain (AWG) of 5.30±0.04 g, the highest specific growth rate (SGR) 3.06±0.01 and lowest feed conversion ratio (FCR) 1.28±0.01was recorded in the treatment contained 1% K in diet (D_K) than control. There is significant ($p<0.05$) difference in AWG, SGR and FCR for *L. vannamei* fed with various levels of minerals incorporated diets compared to control diet fed group.

Keywords: AWG, FCR, mineral diets, *Litopenaeus vannamei*, low salinity, SGR

Introduction

Shrimps are omnivorous organisms, which mean that a part of their natural diet is based on plants. The shrimp culture received maximum importance in the human consumption due to its unique taste, high nutritive value and persistent demand in world market in general. Penaeid shrimps represent one of most attractive species for aquaculture industries in tropical and subtropical regions. It provides high quality rich protein, calcium and various extractable compounds and minerals for human body, while low in calorie and fat [1]. Pacific white leg shrimp, *Litopenaeus vannamei*, is an economically important farm raised shrimp due to its great economic value, rapid growth rate and tolerance of a wide range of salinities and temperatures [2]. Pacific white leg shrimp, *Litopenaeus vannamei* is the popular shrimp species cultured worldwide [3, 4, 5] mainly because of its ability to tolerate a wide range of salinity (0.5 to 40 ppt). In India, after the introduction of *Litopenaeus vannamei* in 2009, farmers are facing mineral deficiency problems especially in low saline water. These problems are being remediated by supplementation of minerals through commercially available mineral mixtures/mineral salts to pond water. However, farmers started adding these mineral mixtures in both high and low salinity cultured ponds without scientific rationale. The data available on the requirement of minerals for shrimp at different salinities is very limited. Besides this, due to lack of awareness about mineral supplementation, shrimp farmers are applying more amount of mineral mixtures to the culture ponds. Many commercial mineral mixtures available in the market are claiming about the supplementation of 3 to 8 minerals and so on. But there is no data on the composition of these minerals and their actual availability in water upon application of mineral mixtures in different saline waters [6].

Shrimp requires water with a specific concentration range of the major anions (bicarbonate, carbonate, sulphate and chloride) and major cations calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) to perform the metabolic and physiological activities. Survival and growth are the essential factor for aquaculture industry. It plays a crucial role, because the primary success of aquaculture is determined by the survival of the cultivable species. The better survival and growth indicates the better quality of water, food and environment.

In aquaculture industry, feed is one of the responsible factors affecting the survival and growth. Minerals (essential elements) are the part of essential micronutrient in aqua feed. An adequate level of mineral requirement is necessary for better survival and growth of shrimps [7]

Minerals are crucial to establish minimum requirement and maximum tolerance for an element to secure optimal health and growth of living organisms. Knowledge about quantitative dietary mineral requirement of crustacean is fragmentary. The dietary sources of some minerals are considered as crucial for their necessary growth and other physiological regulations, especially in crustaceans because of the repeated losses of certain minerals during moulting. For instance, the more soluble minerals such as Ca, P, Na, K and Cl are involved in the maintenance of acid-base balance and membrane potential [8]. Mineral requirement studies normally engage experiments, where animal responses or performance characteristics were studied relative to the feeding graded levels. Although minerals may be present in adequate quantities in feedstuffs for shrimp diets mineral deficiencies can occur under intensive culture conditions. The lack of certain specific minerals may be due to the presence of certain compounds that bind the elemental form of the mineral that is used in the feed, and antagonistic or synergistic reactions in the gastro-intestinal tract are factors that sometimes cause dietary mineral imbalances or deficiencies [7].

Minerals have an important role in shrimp metabolism and growth. Shrimp extracts most of the minerals from the surrounding water, while some are dietary essentials. The mineral profiles of whole shrimp across the wide range of salinities have not yet been reported so far though the effect of mineral supplementation through water [9] and diet [10, 11] were studied in low saline water. Mineral requirements of *L. vannamei* reared in low-salinity water are important for the development of economical feeds and reduction of environmental impact caused by shrimp culture. This study provides baseline data for the identification of nutritional manipulations that may mitigate the variable growth that has plagued low salinity production systems.

Materials and Methods

The experiment was conducted in Wet Laboratory of the Department of Aquaculture, College of Fishery Science, Muthukur, Nellore, Andhra Pradesh. In the present study the plastic tubs were used for the experiments with the size of 0.6x0.3x0.3 m. fifteen plastic tubs were stalked on iron racks. Plastic tubs were located in a secured place where there is no direct sunlight. The tubs were filled with seawater and diluted to required salinity by adding freshwater (15 ppt). Water in the plastic tubs was aerated by using air stones connected to the air compressor. The water in all the tubs was checked for various water quality parameters daily. Five numbers of Shrimps with initial average weights of 20.30±0.02 – 20.31±0.02 g were introduced in to each plastic tubs and triplicates were maintained for each treatment includes control. Regular water exchange of 20% was done every day. Left over feed, excreta and other debris were 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 (table1). Experimental diets were prepared with same ingredients as used in control diet. 1% of vitamin mixture was added to experimental diets. Individual mineral salts like calcium chloride ($CaCl_2$), potassium chloride (KCl), magnesium chloride ($MgCl_2$), and sodium chloride (NaCl) were procured from the market. The concentration of minerals registered in commercial $CaCl_2$, $MgCl_2$, KCl and NaCl salts were 36.11% Ca, 25.52% Mg, 46.68% K and 39.01% Na, respectively. In this study various experimental diets were prepared and their mineral inclusion levels are as follows, Control diet (D_C) and D_{Ca} (2 g Ca), D_{Mg} (200 mg Mg), D_K (10 g K) and D_{Na} (10 g Na) kg^{-1} of feed (table 2).

Table 1: Feed ingredients (%) and Mineral levels added in experimental diets for *L. vannamei*.

Ingredient	Control (C)	D_{Ca}	D_{Mg}	D_K	D_{Na}
Soybean meal	50	50	50	50	50
Fish meal	14	14	14	14	14
Ground nut oil cake	15	15	15	15	15
Deoiled ricebran	14	13.80	13.98	13	13
Maize	6	6	6	6	6
Vitamin and mineral mix %	1	1	1	1	1
Calcium (Ca)	0	0.2	0	0	0
Magnesium (Mg)	0	0	0.02	0	0
Potassium (K)	0	0	0	1	0
Sodium (Na)	0	0	0	0	1

Table 2: Mineral salts added to the experimental diets and concentration of minerals registered in commercial $CaCl_2$, $MgCl_2$, KCl and NaCl salts.

Mineral(gm/Kg)	Commercial mineral salts added to diets					Mineral salts (% Ca, Mg, K, Na)			
	D_C	D_{Ca}	D_{Mg}	D_K	D_{Na}	$CaCl_2$	$MgCl_2$	KCl	$NaCl_2$
Ca	-	5.54	-	-	-	36.11	-	-	-
Mg(mg/kg)	-	-	783.70	-	-	-	25.52	-	-
K	-	-	-	21.42	-	-	-	46.68	-
Na	-	-	-	-	25.64	-	-	-	39.01

Assessment of water quality parameters

Water quality parameters, temperature (Digital pH and temperature meter), pH (Digital pH and temperature meter), dissolved oxygen (Titrimetric Winkler's method) [12] Total hardness, Total alkalinity, Total ammonia and Nitrite were measured in-situ at 9.00 hrs on daily basis.

Growth Performance

The growth parameters were monitored at a regular interval of 7 days for the whole culture period of 28 days. The shrimps were weighed and the growth parameters are calculated with the given formulae.

Weight Gain (g)

WG (g) = Final Weight – Initial Weight

Specific growth rate (SGR)

$$SGR (\%) = \frac{\ln(\text{Final Weight}) - \ln(\text{Initial Weight})}{\text{Experimental period in days}} X100$$

Feed conversion ratio (FCR)

$$FCR = \frac{\text{Feed given (Dry Weight)}}{\text{Body Weight gain (Wet Weight)}}$$

Statistical Analysis

Statistical analyses were performed using Web Agri Stat Package (WASP) version 2.0. The data obtained on Average Weight Gain, SGR and Feed Conversion Ratio was statistically analysed by applying Randomized Block Design (RBD) of two-way classification.

Results and Discussion

Water quality parameters

In the present study important water quality parameters such as Temperature, pH, Dissolved oxygen, Total Hardness, Total alkalinity, Total Ammonia and Nitrite were observed for daily basis (mentioned weekly average values) and sampling values observed in the range presented (table 3). The water quality parameters were similar during the experimental period and maintained in acceptable level for the *L. vannamei* culture.

Table 3: The water quality parameters value ranges during experiment period.

Parameter	Range
Temperature ^{0}C	28.02±0.10 - 30.27±0.21
pH	7.83±0.02 - 8.33±0.02
DO (mg/L)	6.11±0.01-7.14±0.02
Total Hardness (mg/L)	1288.67±1.53 - 1409.33±0.58
Total Alkalinity (mg/L)	265.33±1.53 - 310.67±1.53
Total Ammonia (mg/L)	0.08±0.01 - 0.18±0.01
Nitrite (mg/L)	0.05±0.01 - 0.09±0.01

Growth Performance of *L. vannamei*

The Average weight gain, SGR and FCR data observed weekly for different treatments in salinity of 15 ppt was presented in table no 4 and fig 1. Overall after 28 days, among all treatments, the highest average body weight gain of 5.30±0.04 g in treatment D_K and the lowest average body weight gain of 4.53±0.11 g in control (D_C), the highest Specific Growth Rate was recorded 3.06±0.01 in diet potassium (D_K) and lowest was recorded 3.02±0.01 in control diet (D_C) and the highest feed conversion ratio value of 1.33±0.01 in control diet (D_C) and lowest value of 1.28±0.01 in diet potassium (D_K). Other treatments D_{Na}, D_{Ca} and D_{Mg} shown significantly (p<0.05) different from control.

Average Weight Gain and Specific Growth Rate

Dietary supplementation of ingredients that might improve the osmoregulatory capacity of shrimp have also been examined by a number of researchers as a potential means of improving growth in low salinity waters.

The present study indicated that the dietary supplementation of potassium @ 1% level yielded significantly higher weight gain (5.30±0.04 g), specific growth rate (3.06±0.01) and low feed conversion ratio (1.28±0.01) compared to Ca, Mg, Na and Control.

The results coincide with the [11], they reported that there are no significant differences in growth in the NaCl and magnesium treatments. However, significant differences in growth (P<0.05) were observed when using the 10 g K kg^{-1} (1%) treatment, suggesting that dietary supplementation of potassium may help improve growth of the species in low salinity waters. The growth and physiological response of the test shrimps suggested that diet containing 1.48 g K per 100 g diet improved the growth of *L. vannamei* in low-salinity seawater, and enhanced the physiological acclimation of the organism [13].

The potassium supplementation positively affected the growth of the shrimps reared in low-salinity well water (salinity 4) [14]. Similarly, in *Marsupenaeus japonicus* the dietary level of 0.9 g K/100g (0.9%) of diet improved growth [15]. In *P. monodon* optimum dietary potassium requirement of 1.2%–1.5% showed best Weight gain reared with 21 ppt salinity seawater [16]. Similarly, in the present study at 1% potassium diet, the average body weight gain (5.30±0.04 g) and the specific growth rate (3.06±0.01) significantly higher than Ca, Mg, Na including control. Similar trend in growth enhancement with dietary mineral supplementation was observed by many earlier works in *L. vannamei* [14]. In a field trail supplementation source of chelated potassium improved growth in *L. vannamei* [17].

Feed Conversion Ratio

The present study indicated that the dietary supplementation of potassium @ 1% level yielded significantly low feed conversion ratio (1.28±0.01) compared to Ca, Mg, Na and Control. In *P. monodon* optimum dietary potassium requirement of 1.2%–1.5% showed best FCR reared in 21ppt salinity seawater [16]. The feed conversion ratio (FCR) reduced with the increase of potassium supplementation from 5 g K kg^{-1} (0.5%) to 10 g K kg^{-1} (1%). It may be due to higher potassium supplementation levels with the increase of osmolality and respiration rates of animals and stress condition FCR reduced [18]. Similar results were obtained in our study, that lowest feed conversion ratio (1.28±0.01) in 1% potassium diet (D_K) among all other treatments i.e. Na, Ca, Mg and control.

Table 4: The Average Weight Gain (g) (AWG), SGR and FCR of *L. vannamei* in individual dietary mineral supplementation at low salinity.

Parameter	D_C	D_{Ca}	D_{Mg}	D_K	D_{Na}
Initial	20.31±0.02	20.31±0.02	20.31±0.03	20.30±0.02	20.31±0.02
Final	24.84±0.13	24.93±0.08	25.49±0.06	25.60±0.03	24.91±0.03
AWG	4.53±0.11	4.62±0.07	5.18±0.07	5.30±0.04	4.60±0.04
SGR	3.02±0.01	3.03±0.01	3.05±0.01	3.06±0.01	3.03±0.01
FCR	1.33±0.01	1.31±0.01	1.29±0.01	1.28±0.01	1.31±0.01

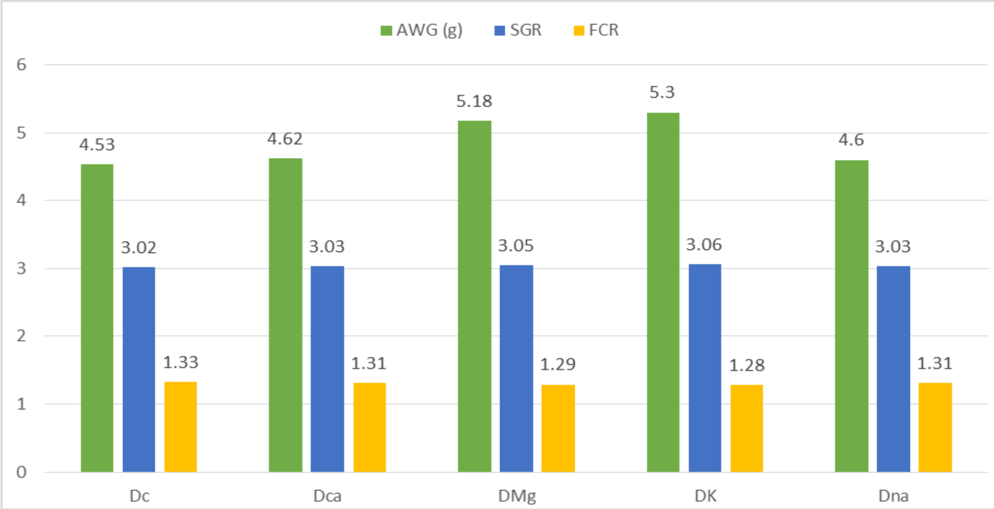

Fig 1: The Average Weight Gain (g) (AWG), SGR and FCR of *L. vannamei* in individual dietary mineral supplementation at low salinity.

Conclusion

It is concluded that the dietary supplementation of 1% potassium diet (D_K) performs better growth in terms of Average weight gain, SGR and FCR of *L. vannamei* among all other treatments in low saline water conditions. However, these results do not justify a recommendation that mineral supplements in feed can completely replace mineral supplements to the culture medium.

Acknowledgement

The authors sincerely thanks to Sri Venkateswara Veterinary University, Tirupati for extending facilities to carry out this work. The Sri Venkateswara Veterinary University, Ph.D. Fellowship granted to the first author, is greatfully acknowledged.

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Supplementation of potassium, magnesium, and sodium chloride in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters. Aquaculture Nutrition. 2007b; 13:104-113.
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