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Growth and carcass mineralisation of Pacific whiteleg shrimp *Penaeus vannamei* Boone 1931 in response to water salinity

R. JANNATHULLA, J. SYAMA DAYAL, V. CHITRA, K. AMBASANKAR AND M. MURALIDHAR
ICAR-Central Institute of Brackishwater Aquaculture, 75, Santhome High Road, RA Puram, Chennai - 600 028 Tamil Nadu, India
e-mail: syamdayal@rediffmail.com

ABSTRACT

The effect of water salinity (3, 10, 20, 30, 40, 50 and 60‰) on growth and proximate as well as mineral composition of Pacific whiteleg shrimp *Penaeus vannamei* was evaluated in a 45-day indoor trial. Significantly higher ($p<0.05$) growth was observed between 10 and 30‰ salinity, with specific growth rate and daily growth coefficient of 2.57-2.64 and 2.00-2.08 respectively. Poor survival of 26.66% was noticed at 60‰. Higher levels of protein and lipid as well as lower levels of moisture and ash were found in shrimp reared in high salinity water. Calcium level in whole shrimp was around 30 g kg^{-1} up to 30‰ and a gradual reduction was observed from 40 to 60‰ (23.49 to16.24 g kg^{-1}). Sodium and potassium contents were significantly ($p<0.05$) higher and lower respectively in hyper saline reared shrimp carcass. Ca: P ratio was almost constant at 3:1 in shrimps reared up to 30‰ salinity and the ratio decreased beyond 30‰. The mineral profiles of water and shrimp were negatively correlated for calcium (-0.830), potassium (-708) and Ca: P ratio (-0.654). The present results indicate that potassium and magnesium supplementation may be helpful in low saline waters and limiting the mineral quantities in the diet especially calcium, magnesium, sodium and potassium may be advised for high salinity shrimp rearing.

Keywords: Ca: Mg ratio, Ca: P ratio, Mineral composition, *Penaeus vannamei,* Pacific whiteleg shrimp, Salinity

Introduction

Shrimp farming, particularly the culture of Pacific whiteleg shrimp *Penaeus vannamei* Boone 1931 has become a fast growing food producing sector, because of its excellent growth performance and tolerance to a wide range of salinity. *P. vannamei* grows well between 0.5 and 40‰ (Saoud *et al*., 2003) by effectively maintaining osmotic homeostasis through ionic regulation, but still problems arise due to variations in the ionic profiles of the pond water. The lack of correct proportion of essential ions in low saline water as well as excess quantity of certain ions in high saline water resulted in reduced growth and survival of shrimp (Saoud *et al*., 2003). In an earlier study carried to understand the role of organic osmolytes, the effect of salinity (3-50‰) on haemolymph free amino acids and metabolic profiles were reported in *P. monodon* (Dayal *et al*., 2013). 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 salinity of source and pond waters fluctuates considerably between wet and dry seasons, induced by heavy rains or floods and drought (Dayal *et al*., 2013). In India, *P. vannamei* is being cultured in very low salinities (~2‰) in Godavari and Krishna districts of Andhra Pradesh and very high salinities (50-60‰) in Tamil Nadu and Gujarat, with varied productivity performances. But, the farmers are applying mineral mixtures irrespective of salinities and ionic profiles, without much scientific basis.

Concentration of the elements stored in the carcass can be influenced by ionic profiles of the culture medium and could be used to ascertain the mineral requirement of cultured species (Baker, 1986). Deficiency or excess of certain minerals in pond water results in improper tissue mineralisation and alterations in metabolic functions, thereby leading to organ malfunctions, which ultimately causes death (Saoud *et al*., 2003; Roy *et al*., 2007). 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 (Pragnell and Fotedar, 2006) and diet (Zhu *et al*., 2006; Roy *et al*., 2007) were studied in low saline water. Hence, the impact of wide range (3-60‰) of salinity and ionic profiles of water medium on growth and mineral profiles of *P. vannamei* was investigated in the present study to remedy the problems due to deficiency and/or excess of ionic profiles. The results obtained will provide the baseline data for dietary manipulations and/or mineral supplementation required according to the pond water salinity/ionic profiles.

Materials and methods

Shrimp husbandry and experimental conditions

Juveniles of *P. vannamei* of average weight 3.04±0.27 g, grown at 20‰ salinity were procured from a farm near to the Muttukadu Experimental Station of the ICAR-Central Institute of Brackishwater Aquaculture (CIBA), Chennai. The animals were acclimatised to experimental salinity by either stepwise increase or decrease at the rate of 2‰ per day from the original salinity, by the addition of sea salt or freshwater, respectively. Upon completion of acclimatisation, all the shrimps were maintained in the same experimental condition for two more weeks.

The juveniles were randomly stocked in experimental tanks (500 l) at the rate of 15 per tank and each treatment was carried out in triplicate. The shrimps in each treatment group were weighed individually after acclimatisation, followed by 24 h starvation. To remove excess moisture, shrimps were blotted with tissue paper and weighed to the nearest 0.001 g using an electronic balance. Shrimps were fed on the basal diet thrice a day at the rate of 6% of total body weight. The proximate and mineral composition of experimental diet is given in Table 1. The amount of diet in relation to survival, body weight and intake was adjusted. Throughout the study period (45 days), 80% water was exchanged every day and water quality parameters were measured periodically. The water temperature, dissolved oxygen and total ammonia-nitrogen were maintained in the range of 26-29°C, 5.5-7.5 mg l^{-1} and <0.1 ppm, respectively. The whole shrimp carcass was analysed for proximate and mineral profiles at the end of the experiment. Growth performance in terms of specific growth rate (SGR), daily growth coefficient (DGC), feed conversion ratio (FCR) and survival was determined as follows.

Table 1. Ingredient and chemical composition of basal diet used in the present study (% on fed basis)

Ingredient composition	Percentage
Fishmeal	20
Acetes	10
Soybean meal	25
Sunflower oil cake	5
Groundnut oil cake	5
Wheat flour	19
Broken rice	10
Fish oil	2
Lecithin	1
Vitamin-mineral mix	2
Binder	1
Proximate composition	
Moisture	9.47
Crude protein	37.49
Ether extract	6.30
Crude fiber	3.14
Nitrogen free extract	30.73
Total ash	12.87
Macro elements	
Calcium	2.77
Magnesium	0.33
Phosphorus	1.89
Potassium	0.85
Sodium	0.67
Ca:P ratio	1.47

Vitamins (g kg^{-1}): Thiamine hydrochloride (25.50 g), Riboflavin (25.00 g), Prydoxine hydrochloride (50.00 g), Cyanocobalamine (0.10 g), Menadione (5.00 g), All-trans tocopherol acetate (99.00 g), Retinyl acetate (10.00 g), Vitamin D (50 g), Nicotinic acid (101.00 g), D-Ca-pantothenate (61.00 g), Biotin (25.00 g), Folic acid (6.25 g), Inositol (153.06 g).
Minerals (g kg^{-1}): Ferric citrate (13.70 g), $ZnSO_4.7H_2O$ (28.28 g), $MgSO_4.7H_2O$ (0.12 g), MnSO4. H2O (12.43 g), $CuSO_4$. 5 H_2O (19.84 g), $CoC_{12}.6H_2O$ (4.07 g), KIO_4 (0.03 g), KCl (15.33 g) and Na_2SeO_3 (0.02 g).

SGR = [ln(Final weight) - ln(Initial weight)] / Days of experiment X 100

DGC = [Final weight$^{1/3}$ - Initial weight$^{1/3}$] / Days of experiment X 100

FCR = Feed intake (g) / Weight gain (g)

Survival (%) = Final number of animals / Initial number of animals X 100

Laboratory analysis

Proximate composition of whole shrimps in terms of moisture, crude protein, ether extract, crude fiber and total ash was analysed as per the method of AOAC (1997). Carcass mineral contents were determined by ICP-OES (Agilent 5100 VDV) after microwave digestion with Anton-Par microwave system using nitric acid and hydrochloric acid. The calibration curve was plotted and checked for linearity at five different concentrations of 2, 4, 6, 8 and 10 mg l^{-1} with 23 element standard mix (Merck, Cat No: 1.11355.0100). The analytical conditions were maintained at 0.6 l min^{-1} nebuliser flow, 0.2 l min^{-1} auxiliary flow and 15 l min^{-1} plasma flow.

Statistical analysis

All the data collected from the entire study were statistically evaluated by one-way analysis of variance (ANOVA) using SPSS/17.0 software and the difference between means was compared by Duncan's multiple range test. Comparison of means was carried out at 5% significance level ($p<0.05$).

Results and discussion

Minerals such as calcium, magnesium, potassium and sodium increased gradually with increase of salinity

in experimental waters. The ratio of Mg: Ca and Na: K was directly proportional to the water salinity (Table 2) and the effect of these ionic changes on growth rate and body compositions are discussed.

Growth indices

The changes in growth performance and feed utilisation of *P. vannamei* juveniles cultured at different salinity are given in Table 3. Shrimps reared between 10 and 30‰ recorded the highest growth performance in terms of specific growth rate (SGR) and daily growth coefficient (DGC) (2.57-2.64 and 2.00-2.08, respectively), compared to both low (3‰) and high (beyond 40‰) salinities. The results are in agreement with the findings of Li *et al*. (2007) in *P. vannamei*. The feed conversion ratio ranged from 1.94 to 2.45, except for the high value of 3.25 at 60‰. Sang and Fotedar (2004) have also observed reduced food assimilation efficiency in shrimp reared at higher salinities.

Even though the present trial was conducted under indoor condition in the absence of natural productivity, shrimp survival was relatively high between 3 and 50‰ (75.53 to 95.53%). This attributes to the overall quality of rearing conditions that were maintained within the optimal range. The decrease in survival was higher at 60‰ (26.66%), indicating the limited capability of *P. vannamei* juveniles to tolerate extremely high salinities. Visual observations showed the dead shrimps to be soft, indicating that they died during or after ecdysis stage which might be due to the wide changes in ionic profiles of water (Davis *et al*., 1993).

Carcass proximate composition

The carcass proximate composition of *P. vannamei* reared at different salinities is presented in Table 4. The moisture content significantly ($p<0.05$) reduced from 78.34 (10‰) to 75.56% (30‰) and did not differ much beyond 30‰ (75.51-75.62%). In general, shrimp catabolises protein and/or lipids (Sang and Fotedar, 2004) to fulfill the energy demand for osmoregulation process during stress condition, if sufficient energy is not provided through the diet. Contrary to the above hypothesis, the energy nutrients like protein and lipid contents of shrimp carcass significantly ($p<0.05$) increased at higher salinities than optimal/lower salinities in the present study. Similar results of higher lipid and/or protein contents have been reported in *P. vannamei* (Huang *et al*., 2004) and crab, *Chasmagnathus granulate* (Luvizotto-Santos *et al*., 2003), which indicated the failure of mobilisation of stored nutrients, especially lipids, in the hyper osmotic stress condition. Ash content in the carcass decreased with increase in salinity beyond 30‰ (Table 4), which might be due to the higher reduction of calcium content (Table 5) in shrimp at hyper salinities as reported by Li *et al*. (2007), in *P. vannamei* exposed to different salinities.

Table 2. Ionic profiles of different saline waters used to culture the juveniles of *Penaeus vannamei* (mg l^{-1})

Minerals	Salinity of experimental water (‰)						
	3	10	20	30	40	50	60
Calcium	147.88	228.88	279.25	314.63	378.13	401.25	409.00
Magnesium	206.42	381.55	678.05	862.55	1240.67	1360.05	1447.80
Potassium	35.25	62.13	128.50	179.00	286.88	330.63	362.88
Sodium	980.13	1857.13	3762.00	6538.25	9368.25	12372	16540.75
Mg:Ca ratio	1.39	1.67	2.43	2.74	3.28	3.38	3.54
Na:K ratio	27.82	29.90	29.28	36.54	32.67	37.41	45.59

All the values are mean of six observations

Table 3. Growth performance of *Penaeus vannamei* juveniles grown in different saline waters

Salinity (‰)	Growth response of *P. vannamei*			
	SGR	DGC	FCR	Survival (%)
3	$2.47^{bc}\pm0.11$	$1.91^{bc}\pm0.12$	$2.27^{bcd}\pm0.60$	$75.53^{b}\pm16.76$
10	$2.63^{a}\pm0.02$	$2.07^{a}\pm0.01$	$2.06^{de}\pm0.47$	$95.53^{a}\pm3.87$
20	$2.57^{ab}\pm0.01$	$2.00^{ab}\pm0.01$	$1.94^{e}\pm0.34$	$91.10^{ab}\pm10.18$
30	$2.64^{a}\pm0.05$	$2.08^{a}\pm0.02$	$2.11^{cde}\pm0.37$	$95.53^{a}\pm3.87$
40	$2.35^{c}\pm0.14$	$1.85^{c}\pm0.14$	$2.45^{b}\pm0.38$	$91.10^{ab}\pm3.81$
50	$2.09^{d}\pm0.07$	$1.62^{d}\pm0.06$	$2.38^{bc}\pm0.27$	$75.55^{b}\pm10.20$
60	$1.04^{e}\pm0.02$	$0.74^{e}\pm0.02$	$3.25^{a}\pm0.09$	$26.66^{c}\pm6.65$

All the values are mean±SD of three observations

Means bearing same superscript in a column do not differ significantly ($p>0.05$)

SGR - Specific growth rate, DGC - Daily growth coefficient, FCR - Feed conversion ratio

Table 4. Carcass proximate composition of *Penaeus vannamei* (% wet basis) grown in different saline waters

Salanity (‰)	Body proximate composition of *P. vannamei*					
	Moisture	Crude protein	Ether extract	Crude fiber	NFE	Total ash
3	76.95^{b}±0.08	16.42^{c} ±0.15	1.04^{d}±0.02	1.43^{b}±0.02	1.46^{c}±0.25	2.70^{d}±0.05
10	78.34^{a}±0.22	15.66^{d}±0.65	1.06^{d}±0.02	1.36^{d}±0.02	0.84^{d}±0.54	2.74^{c}±0.04
20	76.22^{c}±0.09	16.72^{b}±0.82	1.06^{d}±0.04	1.51^{a}±0.01	1.70^{bc}±0.79	2.79^{b}±0.04
30	75.56^{d}±0.08	16.80^{b}±0.56	1.19^{c}±0.03	1.51^{a}±0.01	2.01^{a}±0.59	2.93^{a}±0.04
40	75.62^{d}±0.12	16.91^{b}±0.65	1.50^{a}±0.03	1.31^{c}±0.01	2.16^{a}±0.61	2.50^{f}±0.02
50	75.62^{d}±0.13	16.94^{b}±0.62	1.37^{b}±0.03	1.40^{c}±0.05	2.11^{a}±0.63	2.56^{e}±0.03
60	75.51^{d}±0.20	17.47^{a}±0.83	1.39^{b}±0.02	1.26^{f}±0.01	1.94^{ab}±0.98	2.43^{g}±0.03

All the values are mean±SD of six observations
Means bearing same superscript in a column do not differ significantly (p>0.05)
NFE - Nitrogen free extract

Table 5. Carcass mineral composition (dry matter basis) of *Penaeus vannamei* grown in different saline waters

Salinity (‰)	Carcass minerals content of *P. vannamei*						
	3	10	20	30	40	50	60
Macro elements (g kg^{-1})							
Calcium	32.39^{b} ±0.83	34.04^{a}±0.45	31.09^{c}±1.36	31.84^{bc}±1.23	23.49^{d}±1.62	21.64^{e}±0.38	16.24^{f}±0.45
Magnesium	2.12^{bc}±0.09	2.57^{a}±0.23	2.02^{c}±0.09	2.57^{a}±0.14	2.37^{ab}±0.14	2.07^{c}±0.23	1.67^{d}±0.49
Phosphorus	10.82^{a} ±0.68	11.32^{a}±0.41	10.62^{ab}±0.59	10.57^{ab}±1.61	10.02^{b}±0.09	10.62^{ab}±0.68	9.92^{b}±0.32
Potassium	8.35^{a}±0.42	7.60^{bc}±0.21	7.80^{b}±0.38	7.45^{cd}±0.42	6.85^{e}±0.25	6.85^{e}±0.33	7.25^{d}±0.42
Sodium	7.38^{b}±0.31	7.58^{b}±0.49	6.48^{c}±0.14	6.63^{c}±0.45	5.43^{d}±0.27	7.28^{b}±0.06	8.03^{a}±0.18
Micro elements (mg kg^{-1})							
Copper	73.98^{e}±0.43	76.18^{ed}±0.43	83.43^{a}±1.62	82.88^{a}±0.43	77.83^{c}±0.82	80.68^{b}±0.43	75.63^{de}±3.22
Iron	45.22^{e}±1.24	63.57^{d}±1.64	85.27^{b}±0.54	74.12^{c}±1.24	64.67^{d}±3.23	124.12^{a}±1.24	73.57^{c}±1.64
Manganese	7.78^{abc}±0.58	8.28^{a}±1.21	7.58^{bcd}±0.49	7.48^{cd}±0.23	7.18^{d}±0.40	8.03^{ab}±0.10	8.18^{a}±0.23
Zinc	61.83^{b}±0.45	65.23^{a}±0.61	57.88^{d}±0.83	58.98^{c}±0.83	56.23^{e}±0.45	55.68^{e}±1.62	56.23^{e}±0.45
Ca:P ratio	2.99^{a}±0.11	3.00^{a}±0.07	2.93^{a}±0.29	3.05^{a}±0.36	2.34^{b}±0.17	2.04^{c}±0.16	1.63^{d}±0.09
Ca:Mg ratio	15.23^{a}±0.29	13.28^{b}±1.00	15.38^{a}±1.35	12.36^{b}±0.19	9.94^{c}±1.24	10.50^{c}±0.99	10.39^{c}±2.97

All the values are mean± SD of six observations
Means bearing same superscript in a row do not differ significantly (p>0.05)

Carcass mineralisation

The best growth performance was observed between 10-30‰ (Table 3) and hence 20‰ was taken as optimal salinity. The results of carcass mineral composition (Table 5) revealed that *P. vannamei* strictly regulated almost 30 g kg^{-1} of calcium in the body between 3 and 30‰ salinity for the various physiological processes. The calcium content was found to gradually ($p<0.05$) get reduced by 24-48% between 40 and 60‰ as compared to the optimum treatment (Fig. 1). It clearly indicates that *P. vannamei* excretes body calcium in hyper saline conditions to maintain homeostasis. Stevenson (1985) stated that the crustaceans cultured in freshwater store calcium in tissues and hemolymph for cuticular mineralisation, whereas in the saline waters with higher content of calcium, marine penaeids may not need to store calcium in the body. This could be a possible reason for the difference observed in calcium level among the treatments. These results indicate that calcium supplementation in water is not required in low saline water, because its level (147 mg l^{-1}) was far above the reported value in an earlier trial (63 mg l^{-1}) with *P. vannamei* (Cheng *et al*., 2006).

The gradual increase of magnesium in the experimental waters with increasing salinity was more pronounced next to sodium, but neither water nor dietary magnesium influenced the carcass magnesium content in shrimp up to 50‰ (2.02-2.57 g kg^{-1}). Though magnesium storage was higher between 3-50‰ salinity, it was more pronounced in shrimp reared at 10 and 30‰ (Fig. 1). Lower magnesium storage (1.67 g kg^{-1}) at 60‰ could have impaired the shrimp metabolism, possibly by being inadequate for the physiological demand (Roy *et al*., 2007) as confirmed by the poorest survival at 60‰ (Table 3) in the present study. Gong *et al*. (2004) and Cheng *et al*. (2006) reported a dietary requirement of magnesium (0.26-0.35%) for *P. vannamei* reared in low salinity water and the present value was within this range (Table 1). The present results and published literature suggest that supplementation of dietary magnesium for

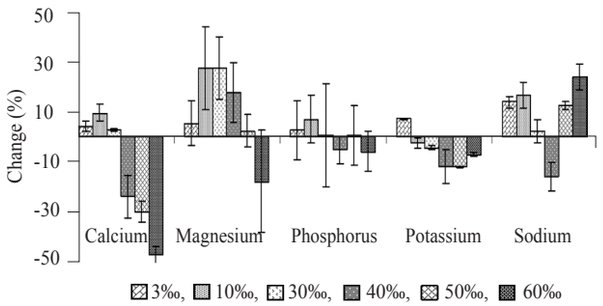

Fig. 1. The percent change in *P. vannamei* carcass major elements among the treatments in comparison with shrimps reared at 20‰ salinity

P. vannamei reared in low saline water would have helped to improve the performance at 3‰ (SGR 2.47) compared to 10-30‰ (SGR 2.57-2.64).

The carcass phosphorus content differed non-significantly among the treatments since absorption of phosphorus is unlike other minerals from the water; thus phosphorus content in feed (Table 1) is exclusively reflected in shrimp (Table 5). The dietary requirement of phosphorus varies widely in the range of 0.35-2.0% for marine penaeids (Kanazawa *et al*., 1984). Davis *et al.* (1993) documented that dietary supplementation of phosphorus had no significant effect on growth in *P. vannamei* reared either in low-saline or in normal seawater. However, reduction in growth was observed at higher levels of dietary phosphorus in *P. monodon* due to a shift in pH of the diet and an interaction with other nutrients (Penaflorida, 1999) and its excretion was linearly related with dietary supplementation (Ambasankar *et al*., 2006), Hence this phenomenon also needs to be considered during supplementation of phosphorus.

In the present study, dietary Ca:P ratio (1.47:1) was within the recommended range (Kanazawa *et al*., 1984). Shrimp maintains a Ca: P ratio of almost 3:1, either by utilising the dietary calcium or by extracting from the rearing medium (Ambasankar *et al*., 2006). Similar values were observed in the present study up to 30‰ salinity and beyond that the ratio gradually decreased due to the reduction of calcium content in shrimp body. Ambasankar *et al.* (2006) reported that dietary phosphorus had a significant effect on phosphorus excretion in *P. monodon.* Hence based on the literature and the present results, it could be concluded that, instead of phosphorus supplementation, limiting the concentration of calcium within the dietary requirement of cultured species would be more beneficial under hyper saline conditions.

The concentration of potassium showed a gradual increase in the experimental waters with increasing salinity, but the same trend was not reflected in carcass composition. Though higher value of potassium was found in shrimp reared at 3‰, difference among the treatments was quite insignificant compared to other macro elements (Fig. 1). This could be due to the proper ionic regulation of potassium by *P. vannamei* (Zhu *et al*., 2006). The potassium content in the diet was 0.85% (Table 1) which was lower than the reported values of 1.2% (Roy *et al.,* 2007) and the supplementation of higher potassium level in the diet would have resulted in better performance at 3‰ (35.25 mg l^{-1}) as suggested by Gong *et al*. (2004). *P. vannamei* has effectively maintained sodium level in the range of 5.43-6.63 g kg^{-1} in 20-40‰ salinity; however, it was not able to osmoregulate efficiently at very low (3-10‰) and high salinity (50-60‰), leading to higher accumulation of sodium (7.28-8.03 g kg^{-1}) which could be attributed to hypo and hyper osmotic stress condition. The present result is in agreement with the findings of Roy *et al*. (2007) in *P. vannamei*, who opined that dietary supplementation of sodium chloride is a remedy for such stress conditions when reared in low saline waters by potentially counteracting losses to the medium. McFarland and Lee (1963) reported the same in *Litopenaeus setiferus*. However, there were no available reports till date regarding the sodium content of shrimp reared in high salinity waters. Though the deposition of sodium was found to be high in most of the treatments than the optimum (Fig. 1), the higher deposition in extreme high saline environment indicates that *P. vannamei* had better homeostasis process and tissue mineralisation for sodium at very low saline conditions compared to extreme saline conditions. The mineral profiles of water and shrimp indicated that the levels of calcium, (-0.830), potassium (-708) and Ca:P ratio (-0.654) were negatively correlated. *P. vannamei* exhibited no uniform trend for micro elements. As the micro elements are needed only in minute quantities, further studies are needed to demonstrate the requirements, deficiency and mineral content in shrimps reared under different saline water environments.

The dietary mineral supplementation has to be adopted according to the ionic profiles of culture medium, since shrimps extract most of the minerals from the rearing medium. From the present investigation, it can be concluded that potassium and magnesium supplementation may help in low saline waters and limiting the mineral quantities in the diet especially calcium, magnesium, sodium and potassium may be advised for high salinity rearing. Avoiding the additional supplementation of phosphorous by restricting the dietary calcium level may be useful in high saline rearing environments. The present study also highlighted the tissue mineralisation *vis-a-vis* salinity, which helps in determining the dietary requirements of minerals for *P. vannamei* reared under wide ranges of salinity as well as in the development of economical and environmental friendly feeds.

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