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RESEARCH PAPER

The Effect of Fortification of Potassium and Magnesium in the Diet and Culture Water on Growth, Survival and Osmoregulation of Pacific White Shrimp, *Litopenaeus vannamei* Reared in Inland Ground Saline Water

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Abstract

A 60 day trial was conducted to evaluate the effect of dietary supplementation of graded levels of potassium (K^+) and magnesium (Mg^{2+}) on *Litopenaeus vannamei* reared using IGSW (inland ground saline water, 10 ppt). Two different types of waters, IGSW without ionic fortification (R-IGSW, R-raw) and IGSW fortified with K^+ & Mg^{2+} (F-IGSW, F-fortified) with equivalent salinity sea water was used. Four experimental diets with graded levels of K^+ & Mg^{2+}: F_1 (5 g KCl & 150 mg $MgCl_2$ kg^{-1} of feed); F_2 (10 g KCl & 300 mg $MgCl_2$ kg^{-1} of feed); F3 (15 g KCl & 450 mg $MgCl_2$ kg^{-1} of feed) and F4 (with no ionic amendment) were tested in the trial, for each of the two types of water. A significant improvement in the growth and survival has been observed in the groups fortified with K^+ & Mg^{2+} in the feed ($P<0.05$) irrespective of the potassium and magnesium levels in the water. No significant difference in osmoregulatory capacity, serum and water osmolality and Na^+ K^+-ATPase activity of gill despite the significant variation ($P<0.05$) in serum K^+, Na^+, Mg^{2+} and Ca^{2+} levels were observed among different treatments. It is concluded that growth and survival of *L. vannamei* can be enhanced by supplementing the deficient ions such as K^+ (5 g kg^{-1}) and Mg^{2+}(150 mg Kg^{-1}) through diet as well as in the water simultaneously.

Keywords: *Litopenaeus vannamei*, inland ground saline water, potassium, magnesium; shrimp.

Introduction

Salinization of land and ground water has been affected a huge area of more than 80 million hectares worldwide (Ghassemi, Jakeman, & Nix, 1995) due to both natural and anthropogenic causes (Bennetts, Webb, Stone, & Hill, 2006) resulting in high water tables, reduced productivity, loss of fertility, and alienation of valuable cultivable land (Smith & Barlow, 1999) thus posing serious threat to agriculture. In India, the salt affected areas have spread into an area of 8. 62 mha (Lakra, Reddy, & Harikrishna, 2014). These water reserves could be potentially used for the production of commercial fish species, rendering a much prospective area in the saline aquaculture. However, the inland saline aquaculture is affected by the imbalance in the ionic composition of water, causing poor growth and mass mortality of fishes. The ionic composition of groundwater normally reflects that of seawater, the exact composition varies both locally and regionally. The quality of inland ground saline water (IGSW) is quite different than natural seawater mainly in ionic composition. Potassium (K^+) concentrations are very low, calcium (Ca^{2+}) levels are high and magnesium (Mg^{2+}) levels are highly variable in inland ground saline water compared to natural sea water at the given salinity (Saoud, Davis, & Rouse, 2003). Potassium is the primary intracellular cation, necessary for the activation of the Na^+-K^+-ATPase (Mantel & Farmer, 1983) which is a key component of extracellular ionic regulation. Magnesium is also an essential mineral needed by crustaceans for normal survival and growth (Roy, Davis, Saoud, & Henry, 2007). The supplementation is performed in order to achieve a standard concentration recommended for respective salinity. The amount of potassium required for standard seawater is 320-340 ppm and magnesium amounts up to 590-600 ppm. The ionic supplementation was found effective in improving the growth and survival of various marine species such as grey mullet, *Mugil cephalus* (Barman, Jana, Garg, Bhatnagar, & Arasu, 2005), mulloway, *Argyrosomus japonicas* (Doroudi, Fielder, Allan, & Webster, 2006), Australian snapper, *Pagrus auratus* (Fielder, Bardsley, & Allan, 2001), western king prawn, *Penaeus latisulcatus* (Prangnell & Fotedar, 2006), black tiger prawn, *Penaeus monodon* (Tantulo & Fotedar, 2006) Pacific white shrimp, *Litopenaeus vannamei* (Roy *et al.*, 2010), seaweed and blue

mussel, *Mytilus edulis* (Dinh & Fotedar, 2016).

The Pacific white shrimp, *L. vannamei* is a brackish water species cultured in coastal areas as a valuable aquaculture commodity with high export demand and has been recognized as a candidate species for inland saline aquaculture (Davis, Saoud, McGraw, & Rouse, 2002). In order to maximize the growth and survival of shrimps, ionic manipulation of raw IGSW with K^+ and Mg^{2+} is done with commercial grade muriate of potash (KCl) and magnesium chloride respectively in commercial inland saline shrimp farms (Roy *et al.*, 2010). Use of fertilizers for the ionic fortification of water in inland saline shrimp aquaculture could be costly and hence dietary supplementation of ions such as K^+ and Mg^{2+} in the feed may be a cost effective (Roy *et al.*, 2010). Therefore, the major limiting minerals in inland saline shrimp culture such as K^+ and Mg^{2+} could be met through diet. Furthermore, growth and survival of marine shrimps reared in low saline waters could be improved by dietary supplementation of minerals (Davis *et al.*, 2002; Roy *et al.*, 2010). The ions supplementation in the feed is more economically feasible method than that of the water, and it can increase the profit upto 15% (Iffat, 2016). The major focus of the present study is to evaluate the growth and survival of *L. vannamei* reared in raw IGSW fed with different dietary potassium and magnesium levels and its feasibility to replace ionic amendment through the water.

Materials and Methods

Experimental Site and Animals

The specific pathogen free (SPF) *L. vannamei* PL_{15} (15 days old post larvae) were airlifted from Geekay Hatcheries, Nellore, Andhra Pradesh, India to the experimental site, Rohtak, Haryana, India, which is a salt affected area. The post larvae (PL_{15}) were reared in earthen ponds (200 m^2) for a period of 30 days at 10 ppt inland ground saline water having K^+ levels 100% equivalence to that of sea water and Ca^{2+}: Mg^{2+} ratio maintained at the level of 1:2.5, in order to obtain juveniles (3.19±0.18 g) for the experiment. The PLs were fed with commercial starter feed crumbles (Avanti Feeds Limited, Andhra Pradesh, India) three to four times a day at 25% biomass. The juvenile shrimps were collected from the pond, acclimatized and nursed in 500 L capacity FRP tanks for a period of 6 days with sufficient aeration and *ad libitum* feeding.

Preparation of Control and Treatment Media

The inland saline water required for the experiment was prepared by following the protocols of Raizada *et al.* (2015). Raw IGSW of 15 ppt from a bore well was pumped in cement tanks and allowed to settle for a week. Further, that water was filtered with a 100 µ filter bag and drawn inside the wet laboratory, and then stored in 12 FRP tanks (1200 L). The water was disinfected by application of bleaching powder (Calcium hypochlorite) at 15 mgL^{-1} and vigorously aerated for at least 48 h before use. The stored water was diluted with fresh water to prepare a salinity of 10 ppt which was constant in all treatment media. Two types of water were used in this experiment (i) raw inland ground saline water (R-IGSW); (ii) fortified inland ground saline water (F-IGSW) having K^+ levels 100% equivalence to that of sea water and Ca^{2+}: Mg^{2+} ratio maintained at the level of 1:2.5. F-IGSW was prepared by amending R-IGSW with commercial grade Muriate of Potash (KCl) and Magnesium Chloride ($MgCl_2.6H_2O$) as per the following formulae based on Davis, Boyd, Rouse, and Saoud (2005). The amount of potassium and magnesium were calculated by the following equations:

$$\text{Quantity of potassium for F – IGSW} = (10.7 \times \text{Salinity}) - \text{Available potassium in R – IGSW}$$

$$\text{Quantity of magnesium for F – IGSW} = (\text{Calcium content} \times 2.5) - (\text{Available magnesium in R – IGSW} \times 3.7)$$

All test and reference water were prepared 15 days prior to the commencement of the trial. The salinity of the water was tested using a handheld refractometer (Atago, Tokyo, Japan) and was counter checked with salinity titration (APHA, 2005). The Na^+ and K^+ levels were analyzed using flame photometry (Microprocessor flame photometer, Model 1382, ESICO, Haryana, India). The total hardness, Ca^{2+} and Mg^{2+} concentrations were analyzed using Ethylene diamine tetra-acetic acid (EDTA) titration (APHA, 2005). The ionic profile of the water was analyzed before the commencement of the experiment. The different experimental media were prepared in the same way if and when necessary.

Preparation of Experimental Diets and Analysis of Proximate Composition

Four experimental feeds were used in this study by amending various levels of KCl (Merck, India) and $MgCl_2$ (Merck, India) as sources of K^+ and Mg^{2+} respectively in the commercial shrimp feed pellets of size 1.2-1.6 mm (CP Aquaculture India Pvt. Ltd., Chennai, India) by gelatin coating method adopted from Piper *et al.* (1982) with slight modification. The various experimental diets prepared and their mineral inclusion levels are as follows, F1 (5 g KCl & 150 mg $MgCl_2\,kg^{-1}$ of feed); F2 (10 g KCl and 300 mg $MgCl_2\,kg^{-1}$ of feed); F3 (15 g KCl and 450 mg $MgCl_2\,kg^{-1}$ of feed); and F4 (0 g KCl and 0 mg $MgCl_2\,kg^{-1}$ of feed). These inclusion levels were selected with reference to the supplementation levels used by Roy, Davis, Saoud and Henry (2007) in the same shrimp species. For preparing 1 kg of feed, required amounts of KCl and

$MgCl_2$ were dissolved in 125 ml distilled water and the solution was boiled. Then 1.5 g of gelatin was slowly dissolved in the boiling solution and allowed to cool to room temperature and the mixture was mixed homogeneously with the commercial feed (CP Aquaculture India Pvt. Ltd., Chennai, India). Proximate analysis of the carcass tissue of shrimps and diets were measured by standard methods (AOAC, 1995) mentioned in Table 1.

A 60 day experiment was conducted to test whether the supplementation of potassium and magnesium through feed to improve the production performance of *L. vannamei* in R-IGSW. Two hundred and forty juveniles of *L.vannamei* (3.19±0.18 g) were distributed randomly in eight distinct experimental groups in triplicate with different combinations of K^+-Mg^{2+} amendment in water and feed, following a completely randomized design. The setup consisted of 24 plastic circular tanks (150 L capacity) covered with perforated lids to avoid jumping out of the shrimps. The various treatment groups are as follows, C (R-IGSW with F4 feed); T1 (F-IGSW with F4 feed); T2 (R-IGSW with F1 feed); T3 (R-IGSW with F2 feed); T4(R-IGSW with F3 feed); T5 (F-IGSW with F1 feed); T6 (F-IGSW with F2 feed) and T7 (F-IGSW with F3 feed) based on the concentrations used by Roy *et al.* (2007). Shrimps were fed at a rate of 3% of body weight, in four equal split doses. However, left over feed and shrimp excreta were siphoned out daily and the constant water level was maintained by adding water from respective storage tanks. In all experimental groups, dissolved oxygen (Winkler's method), pH (digital electronic pH meter), and temperature (mercury thermometer) were measured daily, whereas ammonia, nitrite, total alkalinity, total hardness was monitored twice a week by following the standard methods of APHA (1981).

Growth Performance

The growth parameters were monitored at a regular interval of 10 days for the whole culture period of 60 days. The shrimps were weighed and the growth parameters are calculated with the formulae given by Omitoyi (1999) as follows;

Weight Gain (%)

$$WG\,(\%) = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}} X\,100$$

Specific growth rate (SGR)

$$SGR\,(\%) = \frac{\ln(\text{Final Weight}) - \ln(\text{Initial Weight})}{\text{Experimental period in days}} X\,100$$

Feed conversion ratio (FCR)

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

Feed efficiency ratio (FER)

$$FER = \frac{\text{Net weight gain (Wet Weight)}}{\text{Feed given (Dry Weight)}}$$

Protein efficiency ratio (PER)

$$PER = \frac{\text{Net weight gain (Wet Weight)}}{\text{Protein Fed}}$$

Survival Rate

$$Survival\,(\%) = \frac{\text{Total no. of animals harvested}}{\text{Total no. of animals stocked}} X\,100$$

Osmolality and Osmoregulatory Capacity (OC)

Haemolymph samples were drawn at the end of the trial following the methodology adopted by Antony *et al.* (2015). Shrimp in inter molt stage were selected for collection of serum and were collected randomly from animals within a tank and pooled. Haemolymph (500-800 µL) was collected with a 1.0 mL tuberculin syringe from the junction of the cephalothorax and the abdomen by inserting the needle into the pericardial cavity. The samples were transferred to 1.5 mL Eppendorf tubes and are placed in a refrigerator for clotting (4°C, 15 min). Instantly after clotting, the clot was crushed by a plastic rod and the samples were centrifuged at 2990 x *g* for 30min (Spinwin, MC02, Tarsons, Daihan Scientific, Seoul, Korea) to obtain serum (Tantulo & Fotedar, 2006). The water and serum osmolality (mOsm kg^{-1}) were measured using a cryoscopic osmometer (Osmomat® 030, Gonotec GmbH, Berlin, Germany). The osmoregulatory capacity (OC) of the juveniles in different treatments was calculated by subtracting serum and water osmolality (Lignot, Spanings-Pierrot, & Charmantier, 2000). The remaining serum samples were stored at -20° C until further analysis.

Table 1. Proximate composition of experimental diets (% dry matter basis, Mean ± SE)

Diet	CP[1] (%)	EE[2] (%)	NFE[3] (%)	CF[4] (%)	Ash (%)	DE[5] (Kcal/Kg)
F1	34.84±0.11	4.82±0.01	40.01±0.71	10.22±0.08	11.13^{b}±0.03	338.78±2.52
F2	35.12±0.20	4.64±0.01	38.67± 0.69	9.81±0.07	11.76^{b}±0.01	336.92±3.18
F3	35.34±0.21	4.53±0.01	38.16±0.37	9.74±0.02	12.23^{c}±0.01	334.77±2.36
F4	35.32±0.09	4.51±0.02	39.10± 0.26	10.34±0.06	10.74^{a}±0.14	338.27±2.94

[1]Crude Protein, [2]Ether extract, [3]Nitrogen Free Extract, [4]Crude Fiber, [5]Digestible energy (K cal/ 100g) = (% CP x 4) + (% EE x 9) + (NFE x 4) (Halver, 1976) Values with different superscripts in the same column differ significantly ($p < 0.05$) and values are expressed as mean ± SE

Na^+K^+-ATPase Activity

Na^+ K^+ -ATPase activity in gills was determined by modified method of Post and Sen (1967) The reaction mixture consisted of 1ml Tris HCl buffer, 100mM NaCl, 20Mm KCl, 3mM $MgCl_2$, 5mM ATP and the samples were run with and without the presence of ouabain. After the completion of reaction supernatant was taken for the estimation of inorganic phosphorus. The inorganic phosphorus was estimated by the method Fiske and Subbarow (1925). The ATPase activity was expressed as nano moles Pi released $min^{-1}mg^{-1}$ protein at 37°C.

Serum Ionic Concentration

The serum samples were subjected to acidic digestion in microwave digestion system (Microwave Digestion System, Model STARTD, SN-135177, Milestone, USA). The Nitric acid (HNO3, AS008, 70%, Himedia Laboratory Pvt. Ltd., Mumbai, India) and Hydrofluoric acid (HF, AS005, 40%, Himedia Laboratory Pvt. Ltd., Mumbai, India) were added in 5:1 ratio kept for digestion, and completely digested samples were allowed to cool to room temperature; then, digested samples were filtered with Whatman paper of 0.45-µm pore size, and total volume of 50 ml was made with TDW and further subjected to metal analysis through Inductively Coupled Plasma - Atomic Emission Spectroscopy, ICP-AES (SPECTRO Analytical Instruments GmbH, Germany) (Thompson & Walsh, 1989; Moore, 1989) at the SAIF, Indian Institute of Technology, Mumbai, India.

Statistical Analysis

The data was analyzed with a statistical package SPSS version 22.0 in which data were subjected to one-way Analysis of Variance (ANOVA). The difference between the FIGSW and RIGSW was tested with two sample t test after assuming equal variance using Levene's test. The data of various parameters were tested for their normality using shapiro-wilk test. Duncan's multiple range tests were used to determine the significant differences between the means. P-value < 0.05 was considered as statistically significant.

Results

Water Quality and Proximate Composition

The proximate composition of various experimental feed F1, F2, F3 and F4 are given in Table 1. Throughout the 60 day trial, there were no differences in water quality observed among the different experimental treatments. Dissolved oxygen (7.8 mgL^{-1}), pH (8.2), temperature (23.1°C), total ammonia nitrogen (0.25 - 0.36 mgL^{-1}), nitrite nitrogen (0.07 to 0.71 mgL^{-1}), K^+ (12.75- 330 mgL^{-1}), Mg^{2+} (452.5-597.5 mgL^{-1}), Ca^{2+} (86.5-117.5 mgL^{-1}) remained within acceptable limits for the culture of *L. vannamei* (Table 2)

Growth Rate and Survival

There was a significant difference in the survival rates of *L.vannamei* juveniles reared in different treatment groups (Table 3). The survival (%) in the F-IGSW was significantly higher than that of R-IGSW (Table 4). It is to be noted that, all the animals died within 3 days from the start of the experiment in control group (C), in which the experimental animals were reared in raw IGSW by using F4 feed which had no potassium and magnesium supplementation. Except for survival, this treatment has been excluded from the study and further analysis due to complete mortality. Significantly higher survival ($P<0.05$) rates were observed in T5 and T6 groups compared to other treatment groups.

At the end of the experimental trial, there was a significant difference ($P<0.05$) among the various

Table 2. Physico-chemical parameters for growth experiment with juvenile *L. vannamei* (Mean ±S. E)

Water quality parameters	R-IGSW[1]	F-IGSW[2]	NSW[3]
Dissolved Oxygen (mgL^{-1})	7.8±0.01	7.8±0.01	7.8±0.01
Temp (^0C)	23.1±0.03	23.1±0.03	23.1±0.03
Free CO_2 (mgL^{-1})	Nil	Nil	Nil
pH	8.2±0.03	8.2±0.03	8.2±0.03
Hardness (mgL^{-1})	2460±0.02	2793.5±0.12	2488±0.01
Ca^{2+} cont. (mgL^{-1})	107^b±0.03	86.5^a±0.02	117.5^b±0.04
Mg^{2+} cont. (mgL^{-1})	452.5^a±0.04	533.5^b±0.02	597.5^b±0.04
Alkalinity (mgL^{-1})	325.2^b±0.01	290.3^a±0.01	328.7^b±0.01
Ammonia-N (mgL^{-1})	0.25±0.02	0.36±0.02	0.32±0.02
Nitrite-N (mgL^{-1})	0.07±0.01	0.71±0.02	0.49±0.01
K^+ content (mgL^{-1})	12.75^a±0.01	105^b±0.01	330^c±0.01
Na^+ content (mgL^{-1})	3290^a±0.01	3045^a±0.03	3567^b±0.03

[1] Raw Inland Ground Saline Water, [2] Fortified Inland Ground Saline Water, [3] Natural Seawater. Values with different superscripts in the same row differ significantly (P < 0.05) and data is expressed as Mean ±S. E (n=9).

Table 3. Survival, WG[1], SGR[2], FCR[3], PER[4] and Na^+ K^+-ATPase activity levels (mean±SE) of shrimp reared in different treatments after 60 days.

Treatment	Type of water	K^+ Mg^{2+} fortification in feed	Survival (%)	WG (%)	SGR	FCR	PER
C1	R-IGSW	F4 feed (with no ionic amendment)	0.00^{a}	-	-	-	-
T1	F-IGSW	F4 feed (with no ionic amendment)	83.30^{d}±1.08	213.53^{c}±9.90	0.82^{b}±0.02	1.5^{ab}±0.04	1.96^{b}±0.05
T2	R-IGSW	F1 feed (5 g KCl & 150 mg $MgCl_2$ kg^{-1} of feed)	66.93^{b}±0.41	144.46^{ab}±13.46	0.64^{a}±0.04	1.8^{bc}±0.15	1.61^{ab}±0.13
T3	R-IGSW	F2 feed (10 g KCl & 300 mg $MgCl_2$ kg^{-1} of feed)	76.26^{c}±0.40	149.73^{ab}±17.48	0.65^{a}±0.04	1.8^{bc}±0.09	1.60^{ab}±0.13
T4	R-IGSW	F3 feed (15 g KCl & 450 mg $MgCl_2$ kg^{-1} of feed)	66.83^{b}±0.85	124.25^{a}±14.08	0.58^{a}±0.04	2.0^{c}±0.14	1.41^{a}±0.09
T5	F-IGSW	F1 feed (5 g KCl & 150 mg $MgCl_2$ kg^{-1} of feed)	*96.93^{f} ±0.75*	192.44^{bc}±3.07	0.77^{b}±0.07	1.6^{ab}±0.02	1.76^{bc}±0.02
T6	F-IGSW	F2 feed (10 g KCl & 300 mg $MgCl_2$ kg^{-1} of feed)	97.50^{f}±0.30	241.87^{c}±20.94	0.88^{b}±0.04	1.3^{a}±0.04	2.00^{c}±0.05
T7	F-IGSW	F3 feed (15 g KCl & 450 mg $MgCl_2$ kg^{-1} of feed)	90.20^{e}±3.7	204.93^{c}±23.98	0.80^{b}±0.05	1.5^{ab}±0.05	1.82^{bc}±0.09

[1] weight gain; [2]specific growth rate; [3]feed conversion ratio; [4] protein efficiency ratio.
Values with different superscripts in the same column differ significantly (P<0.05) and expressed as mean ± SE (n=9).
*All the animals died within 3 days from the start of the experiment in control group (C)

Table 4. The growth and osmoregulatory responses of juvenile *L. vannamei* in R-IGSW and F-IGSW

Parameters	R-IGSW[1]	F-IGSW[2]
Survival (%)	52.50^{a}± 7.64	91.98^{b}± 3.33
WG (%)	139.48^{a}±7.76	213.19^{b}±10.49
SGR	0.62^{a}±0.02	0.81^{b}±0.02
FCR	1.86^{a}±0.07	1.48^{b}±0.06
PER	1.54^{a}±0.07	1.88^{b}±0.06
Serum K^+ (ppm)	2.42±0.13	3.08±0.33
Serum Na^+ (ppm)	66.03±2.28	79.30±6.39
Serum Ca^{2+} (ppm)	6.00±0.15	7.29±0.61
Serum Mg^{2+}(ppm)	1.03±0.11	1.36±0.20
Serum osmolality (mOsmolkg^{-1})	878.10±1.22	878.33±1.0
Water osmolality (mOsmolkg^{-1})	238.32±0.33	237.24±0.86
Osmoregulatory capacity (mOsmol kg^{-1})	639.33±1.25	638.245±1.64
Gill Na^+K^+-ATPase activity (Nano moles Pi mg^{-1}protein hr^{-1})	11.27±0.26	12.51±0.52

[1] Raw Inland Ground Saline Water, [2] Fortified Inland Ground Saline Water. Values with different superscripts in the same row differ significantly (P<0.05) and data is expressed as Mean ±S. E (n=9).

treatments in terms of WG (%) (Table 3), with highest weight gain (%) observed in T1, T6 and T7 groups compared to other treatments. In this trial, significant differences in SGR, FCR and PER values were observed among various treatment groups (Table 3). The WG (%), SGR and PER values were significantly higher in F-IGSW when compared to those of R-IGSW while FCR values were significantly lower in the F-IGSW group (Table 4). The SGR values were significantly higher (P<0.05) in T1, T5, T6 and T7 groups compared to other treatments. FCR was found to be significantly (P<0.05) lower in the T6 group, compared to other groups. In addition, the PER values were significantly higher in the T6 group compared to the other treatment groups

Parameters of Osmoregulation

There was no significant difference between F-IGSW and R-IGSW groups in terms of osmoregulatory parameters (Table 4). There was no significant difference (P>0.05) among the osmolality of the shrimps reared in different treatment groups (Table 5) with or without potassium magnesium fortification either in water or feed. Osmoregulatory capacity did not vary significantly (P<0.05) among the treatments while it differed numerically within a narrow range (641.33 to 633.33 mOsmol kg^{-1}). A similar trend was observed with serum and water osmolality values. Similarly, there were no significant differences between the Na^+ K^+ -ATPase activity of gills among the treatment groups.

The serum K^+ and Na^+ levels were significantly enhanced by the fortification of potassium in both water and feed. However, the serum K^+ and Na^+ levels were higher in those groups reared in fortified waters (T5, T6 and T7) meanwhile exhibiting a minimal increase with dietary supplementation as highest values were in T7 group. At all the levels of the fortification either in feed or water, the serum Ca^{2+} and Mg^{2+} levels were also increased in the treatments having R-IGSW (T5, T6 and T7) compared to those

Table 5. Serum K^+, Na^+, Ca^{2+}, Mg^{2+}, Osmolality of serum and water and osmoregulatory capacity (OC) (mean±SE) of different treatments

Treatments	Serum K^+ (ppm)	Serum Na^+ (ppm)	Serum Ca^{2+} (ppm)	Serum Mg^{2+}(ppm)	Serum osmolality (mOsmolkg^{-1})	Water osmolality (mOsmolkg^{-1})	Osmoregulatory capacity (mOsmol kg^{-1})	Gill Na^+K^+-ATPase activity (Nano moles Pi mg^{-1}protein hr^{-1})
T1	2.18^a±0.01	60.58^a±0.01	5.54^a±0.03	0.78^a±0.02	875.33±5.36	235.66±1.20	639.66±5.48	13.26±0.24
T2	2.26^a±0.01	61.66^a±0.01	5.71^a±0.03	0.89^a±0.02	875.66±1.45	238.66±1.70	637.01±2.51	11.37±0.20
T3	2.31^a±0.05	67.08^b±0.02	6.08^b±0.02	0.94^a±0.03	879.33±0.57	237.66±0.88	641.33±0.33	11.69±0.59
T4	2.70^b±0.02	69.37^c±0.10	6.22^b±0.05	1.27^b±0.01	879.33±0.33	238.66±1.70	639.66±1.85	10.77±0.06
T5	3.09^c±0.14	83.63^d±0.03	7.53^c±0.01	1.41^c±0.03	879.33±1.85	238.00±3.05	639.66±1.20	11.13±0.43
T6	3.31^d±0.05	83.54^d±0.07	7.67^c±0.04	1.55^d±0.01	879.33±4.91	239.33±2.84	633.33±3.17	13.41±0.44
T7	3.75^a±0.20	89.46^a±0.21	8.42^c±0.02	1.70^a±0.01	879.33±0.88	236.00±2.00	640.33±2.02	12.25±1.24

Values with different superscripts in the same column differ significantly ($P<0.05$) and expressed as mean ± SE (n=9)

groups reared in F-IGSW.

Discussions

In this study, complete mortality of *L. vannamei* juveniles were observed in raw inland ground saline water without fortified feed, which is in agreement with earlier studies (Tantulo & Fotedar, 2007). It might be due to the deficiency of potassium in the water as well as feed. The survival rates of *L. vannamei* were observed to be dependent on the availability of K^+ and Mg^{2+} minerals in low saline waters and dietary amendment of these minerals can significant help to improve the survival of shrimps grown in low saline conditions (Roy, 2006; Shiau & Hsieh, 2001). Under low saline conditions, supplementation of 1% chelated potassium through diet improved the growth of *L. vannamei*; however the survival of animals was comparatively lower (Saoud, Roy, & Davis, 2007). This result is comparable with the present study, in which highest survival was obtained at F1 and F2 feed supplementation in T5 (96.93±0.75) and T6 (97.5±0.30) treatments.

The present study indicates that highest growth of *L. vannamei* can be achieved by minerals fortification in both water and feed. It was also observed that shrimp are able to survive and grow only by providing dietary fortification of potassium-magnesium. Those shrimps reared in fortified water with amended various levels of potassium-magnesium showed best growth performance in terms of weight gain (%) and specific growth rate compared with other treatments. The better FCR and PER were also observed in the groups fed with moderate level of K^+ and Mg^{2+} supplementation (F2 feed) in both R-IGSW and F-IGSW. However, the maximum growth can be achieved only through the fortification of the minerals in the culture system. These results are comparable with the studies of Roy (2006), who observed an enhanced growth of *L. vannamei* reared in low saline water when fed with diet containing potassium amino acid complex (1% K). The diets contain varying concentration of potassium levels from 5-15g Kg^{-1} and the raw and fortified seawater contain 12.75 and 105 mgL^{-1} respectively (Table 2) while seawater concentration of potassium can elevate upto 330 mgL^{-1}. It is reported that dietary potassium exerts a positive effect on the growth, osmoregulation, nitrogen metabolism and immunity of pacific white shrimp reared in low saline seawater (Liu *et al.*, 2014). Gong, Jiang, Lightner, Collins, & Brock (2004) concluded that an increased growth of *L. vannamei* fed a diet containing KCl, MgO, and NaCl compared with a diet without any of these minerals. Reduction in magnesium causes a reduction in potassium also in the body of shrimp, which was reported earlier in *L.vannamei* (Davis, Lawrence, & Gatlin, 1992). In addition to this, magnesium deficiency affected the survival and growth performance of post larva and juvenile shrimps (Saoud *et al.*, 2003).

There were no significant differences between the osmoregulatory capacity (OC) of the shrimps reared in different treatments with or without potassium-magnesium fortification either in water or feed was observed in this study. This may be due to the ability of the animal to cope up within the narrow range of the different ion concentration as described by an earlier study (Gong *et al.*, 2004).

There were no significant differences between serum and water osmolality between the treatments. This would appear to confirm reports of (Roy *et al.*, 2007) who identified a non-significant difference between serum osmolality and concluded that serum osmolality is not affected by dietary supplementation of ions because it is a function of the salinity of the rearing water. Gong *et al.* (2004) reported that dietary supplementation of the sources of KCl, MgO, NaCl, phospholipids, and cholesterol had improved the osmoregulatory capacity of the shrimp.

Ionic regulation and osmoregulatory process is significantly regulated by Na^+ K^+-ATPase activity in crustaceans (Perry, 1997) and potassium is a mandatory for activation of Na^+ K^+ -ATPase (Mantel & Farmer, 1983). In the present study, there is no significant difference in Na^+ K^+ -ATPase activity of gill tissue among the treatments, which is comparable with the studies of Hurtado *et al.* (2006) who concluded Na^+ K^+ -ATPase activity is not affected by either diet or salinity. In contrast, it was demonstrated that the composition of ions in the serum varied in accordance with the dietary levels of minerals and this regulation pattern had been noticed among other penaeid species also (Dall & Smith, 1981; Cheng & Liao, 1986). However, the individual differences in the ionic composition is observed among the groups, indicating that the addition of K^+ and Mg^{2+} ions in the culture water as well as the feed enhanced the K^+, Na^+, Mg^{2+} and Ca^{2+} levels in serum. The groups which received the supplementation by both the water and diet had maximum level of these serum ions. Surprisingly, these enhanced individual ions failed to produce any increments in plasma osmoregulatory capacity which might be due to the fact that the animal have balanced the osmolality with the organic osmolytes (Gong *et al.*, 2004).

Conclusions

The present study demonstrated that *L. vannamei* could be cultured in raw inland ground saline water by supplementing the potassium and magnesium in the feed at a dosage of 10 g KCl & 300 mg $MgCl_2$. However, the survival and growth of the shrimps achieved by ion fortification in water is still higher than those achieved by dietary supplementation. This may be due to the limited ability of the intestine for osmotic regulation compared to gill and skin. However, the similar

osmolalities were achieved by dietary supplementation of ions compared to water supplementation. Our study further explains that the simultaneous supplementation of water and feed (5 g Kg^{-1} KCl & 150 mg Kg^{-1} $MgCl_2$) with the ions yield best growth in *L. vannamei.* Future studies are necessary to optimize the levels of dietary supplementation in order to achieve similar production performance at par with water supplementation of ions.

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