

# Supplementation of potassium, magnesium and sodium chloride in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters

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## Abstract

The culture of *Litopenaeus vannamei* in inland low salinity waters is currently being practiced in various countries around the world. These environments are often deficient in key ions essential for normal physiological function, including potassium (K<sup>+</sup>) and magnesium (Mg<sup>2+</sup>). Farmers have sometimes been able to counteract ionic deficiencies in the water profile by adding mineral salts containing sources of K<sup>+</sup> and Mg<sup>2+</sup>. The purpose of this study was to explore the possibility of correcting deficiencies of K<sup>+</sup> and Mg<sup>2+</sup> in the water profile with dietary supplementation of these minerals. Two separate 7-week experiments were conducted in 4.0 g<sup>-1</sup> artificial low salinity water to evaluate the effects of mineral supplements (K<sup>+</sup>, Mg<sup>2+</sup> and NaCl) to diets of *L. vannamei* reared in low salinity waters. In trial 1 seven diets were formulated (10 g NaCl kg<sup>-1</sup>, 20 g NaCl kg<sup>-1</sup>, 150 mg kg<sup>-1</sup> Mg<sup>2+</sup>, 300 mg kg<sup>-1</sup> Mg<sup>2+</sup>, 5 g K<sup>+</sup> kg<sup>-1</sup>, 10 g K<sup>+</sup> kg<sup>-1</sup>, and a basal diet to serve as a control). Minerals were added in the form of purified potassium chloride (KCl), magnesium chloride (MgCl<sub>2</sub>·6H<sub>2</sub>O) and NaCl. Trial 2 evaluated the use of a coating agent for the Mg<sup>2+</sup> and NaCl treatments, while a K<sup>+</sup> amino acid complex was utilized in the K<sup>+</sup> treatments to reduce mineral leaching. Trial 2 was performed using similar treatment levels as trial 1. Shrimp survival and growth were assessed in both experiments. Results from trial 1 indicated no significant differences in survival, growth or percent weight gain. Results from trial 2 revealed no significant differences in survival and growth in the NaCl and Mg<sup>2+</sup> treatments. However, significant differences in growth ( $P < 0.05$ ) were observed when using the 10 g K<sup>+</sup> kg<sup>-1</sup> treatment, suggesting that dietary supplementation of a K<sup>+</sup> amino acid complex may help improve growth of the species in low salinity waters.

**KEY WORDS:** low salinity water, magnesium, minerals, Pacific white shrimp, potassium, shrimp

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## Introduction

The inland culture of shrimp, particularly the Pacific white shrimp, *Litopenaeus vannamei*, is becoming more widespread throughout the world. Depending on their source, inland waters available for shrimp culture can be of different salinities, and therefore possess different ionic compositions (Boyd & Thunjai 2003). Consequently, although *L. vannamei* can tolerate a wide range of salinities (0.5–60 g L<sup>-1</sup>), aquaculturists still face problems due to mineral deficiencies in the ionic profiles of pond waters (Atwood *et al.* 2003; Saoud *et al.* 2003). The lack of an optimal mix of essential ions, such as K<sup>+</sup> and Mg<sup>2+</sup> has been shown to limit growth and survival of shrimp postlarvae (PL) at acclimation (Saoud *et al.* 2003) as well as during growout (Davis *et al.* 2005).

Alabama has several saltwater aquifers (Feth 1970) that are being utilized as sources of low salinity water for aquaculture (Davis *et al.* 2002). Farmers in west Alabama have been successful in raising *L. vannamei* in inland low salinity waters by raising the K<sup>+</sup> and Mg<sup>2+</sup> levels of their pond waters to more ideal levels. McNevin *et al.* (2004) observed increased shrimp production in Alabama low salinity waters by raising the levels of K<sup>+</sup> (6.2 mg L<sup>-1</sup>) and Mg<sup>2+</sup> (4.6 mg L<sup>-1</sup>) to 40 and 20 mg L<sup>-1</sup>, respectively. Such water treatment using muriate of potash and potassium-magnesium sulphate modified ionic compositions to levels similar to dilute seawater. While the usefulness of supplementing

minerals to inland low salinity well-waters to improve growth and survival has been substantiated, there still exists little information on whether or not dietary supplements of these minerals could also play a role in improving growth and survival of *L. vannamei*.

Shiau & Hsieh (2001) observed benefits of dietary supplements of  $K^+$  for *Penaeus monodon* reared in brackish water. In Arizona, Gong *et al.* (2004) observed increased production through dietary addition of  $K^+$ ,  $Mg^{2+}$ , NaCl, phospholipids and cholesterol to a diet formulated for shrimp cultured in low salinity water. A dietary addition of NaCl has also been reported to provide benefits to euryhaline fish reared at low salinities (Gatlin *et al.* 1992).

Dietary supplementation of key minerals could potentially prove more cost-effective than adding large amounts of agricultural fertilizers to improve ionic profile of inland low salinity waters at inland shrimp farms. The primary objective of the present study was to explore the possibility of remedying ionic deficiencies in the water profile through dietary supplementation of these minerals. Two separate studies were conducted evaluating the supplementation of  $K^+$ ,  $Mg^{2+}$  and NaCl in practical diets of *L. vannamei* reared in low salinity waters.

## Materials and methods

### Culture conditions

The study was conducted at the North Auburn Fisheries Research Station in Auburn, Alabama. Postlarval *L. vannamei* for trial 1 were obtained from GMSB Shrimp Hatchery (Summerland Key, Florida), while shrimp utilized in trial 2 were obtained from Harlingen shrimp farm (Bayview, Texas). Postlarvae were acclimated down to low salinity water ( $4.0 \text{ g L}^{-1}$ ) over a period of 8 h and maintained in a 220-L polyethylene nursery tank connected to a biological filter. During the first week PL were offered a combination of artemia nauplii (200 nauplii per shrimp) and a commercial feed, PL Redi-Reserve (Ziegler Bros, Gardner, PA, USA) at 25–50% body weight. Thereafter, shrimp were offered a commercial feed (Rangen 350 g protein  $\text{kg}^{-1}$ , Buhl, ID, USA) and reared in the nursery system until they were of appropriate size for commencement of growth trials. Both experiments were conducted in 60-L aquaria within a 2400-L recirculating system. Low salinity water comparable in ionic profile to well waters in west Alabama was prepared 2 weeks prior to the commencement of each experiment by adding calcium ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) to  $0.5 \text{ g L}^{-1}$  reconstituted seawater (Crystal Sea Salt, Baltimore, MD, USA). Salinity was then

raised to  $4.0 \text{ g L}^{-1}$  using agricultural grade NaCl. The water reconstituted in the present experiment mimicked the composition of one of the waters in west Alabama where a shrimp aquaculturist was experiencing high mortality. The experimental water was analysed for major ions by inductively coupled argon plasma spectrophotometry (Table 1) according to standard protocols (Clesceri *et al.* 1998). In both experiments, temperature was maintained at approximately  $27.0 \text{ }^\circ\text{C}$ . Light regime was set at 16 h day and 8 h night. Dissolved oxygen, pH, salinity and temperature were measured daily, whereas ammonia (Solorzano 1969) and nitrites (Parsons *et al.* 1985) were measured twice weekly. Water quality parameters were maintained within acceptable limits for *L. vannamei* (Table 2) throughout the experiments.

### Trial 1

The basal diet and six test diets (Table 3) were formulated by substituting an equal weight of cellulose with selected ACS grade mineral supplements. Seven diets were formu-

**Table 1** Ionic composition ( $\text{mg L}^{-1}$ ) of artificial low salinity waters ( $4.0 \text{ g L}^{-1}$ ) used to culture *L. vannamei* at North Auburn Research Station. Composition of seawater is added for comparison

Minerals ( $\text{mg L}^{-1}$ )	Trial 1	Trial 2	Seawater ( $34.0 \text{ g L}^{-1}$ ) <sup>1</sup>
Na	832	1407	10500
K	17	35	380
Mg	21	36	1350
Ca	41	80	400
P	3.5	5.3	–
Zn	0.0	0.0	0.005–0.014
Fe	0.0	0.0	0.002–0.02
Cu	0.0	0.0	0.001–0.09
Mn	0.0	0.0	0.001
Ratios			
Na : K	49 : 1	40 : 1	28 : 1
Ca : K	2.4 : 1	2.3 : 1	1 : 0.95
Mg : Ca	1 : 2	1 : 2	3.4 : 1

<sup>1</sup> Goldberg (1963).

**Table 2** Water quality parameters for growth trials with juvenile *L. vannamei* reared in low salinity waters

Parameter	Trial 1	Trial 2
Dissolved $\text{O}_2$ ( $\text{mg L}^{-1}$ )	$6.8 \pm 0.3$	$7.1 \pm 0.5$
Temperature ( $^\circ\text{C}$ )	$28.8 \pm 1.0$	$28.2 \pm 1.0$
Salinity ( $\text{g L}^{-1}$ )	$4.1 \pm 0.7$	$4.3 \pm 0.1$
pH	$7.7 \pm 0.4$	$8.1 \pm 0.1$
TAN ( $\text{mg L}^{-1}$ )	$0.03 \pm 0.04$	$0.14 \pm 0.08$
$\text{NO}_2$ ( $\text{mg L}^{-1}$ )	$0.06 \pm 0.01$	$0.13 \pm 0.13$

Values represent the mean  $\pm$  standard deviation.

**Table 3** Composition (g kg<sup>-1</sup> dry weight) of the basal diets formulated to contain 350 g protein kg<sup>-1</sup> and 80 g lipids kg<sup>-1</sup> and used in the two growth trials

Ingredient	Basal diet
Fish meal <sup>1</sup>	30.0
Poultry meal <sup>2</sup>	153.0
Soybean meal <sup>3</sup>	336.0
Menhaden fish oil <sup>4</sup>	45.2
Wheat starch <sup>5</sup>	137.8
Whole wheat <sup>5</sup>	196.0
Trace mineral premix <sup>6</sup>	5.0
Vitamin premix <sup>7</sup>	18.0
Stay C <sup>8</sup>	0.7
Calcium phosphate <sup>5</sup>	8.0
Cellufil <sup>9</sup>	20.0
Gelatin <sup>9</sup>	40.0
DL-methionine <sup>5</sup>	1.3
Lecithin <sup>10</sup>	5.0
Cholesterol <sup>10</sup>	2.0
Choline chloride <sup>9</sup>	2.0

<sup>1</sup> Special Select™; Omega Protein Inc., Hammond, LA, USA.

<sup>2</sup> Griffin Industries, Inc., Cold Springs, KY, USA.

<sup>3</sup> De-hulled solvent extracted soybean meal; Southern Sates Cooperative Inc., Richmond, VA, USA.

<sup>4</sup> Omega Protein Inc., Reedville, VA, USA.

<sup>5</sup> MP Biochemicals Inc., Aurora, OH, USA.

<sup>6</sup> g 100<sup>-1</sup> g premix: cobalt chloride 0.004, cupric sulphate pentahydrate 0.250, ferrous sulphate 4.0, magnesium sulphate heptahydrate 28.398, manganous sulphate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulphate heptahydrate 13.193, filler 53.428.

<sup>7</sup> g kg<sup>-1</sup> premix: thiamin HCl 0.5, riboflavin 3.0, pyridoxine HCl 1.0, DL Ca-Pantothenate 5.0, nicotinic acid 5.0, biotin 0.05, folic acid 0.18, vitamin B<sub>12</sub> 0.002, choline chloride 100.0, inositol 5.0, menadione 2.0, vitamin A acetate (20 000 IU g<sup>-1</sup>) 5.0, vitamin D3 (400 000 IU g<sup>-1</sup>) 0.002, DL- $\alpha$ -tocopheryl acetate (250 IU g<sup>-1</sup>) 8.0, Alpha-cellulose 865.266.

<sup>8</sup> 250 mg kg<sup>-1</sup> active C supplied by Stay C® (L-ascorbyl-2-polyphosphate 25% Active C); Roche Vitamins Inc., Parsippany, NJ, USA.

<sup>9</sup> ICN, Aurora, OH, USA.

<sup>10</sup> Fisher Scientific, Pittsburgh, PA, USA.

lated to contain 360 g protein kg<sup>-1</sup> and 80 g lipid kg<sup>-1</sup>. Treatment diets contained one of the following minerals: 10 g NaCl kg<sup>-1</sup>, 20 g NaCl kg<sup>-1</sup>, 150 mg kg<sup>-1</sup> Mg<sup>2+</sup> (0.6 g kg<sup>-1</sup> MgCl<sub>2</sub>·6H<sub>2</sub>O), 300 mg kg<sup>-1</sup> Mg<sup>2+</sup> (1.2 g kg<sup>-1</sup> MgCl<sub>2</sub>·6H<sub>2</sub>O), 5 g K<sup>+</sup> kg<sup>-1</sup> (9.6 g kg<sup>-1</sup> KCl) and 10 g K<sup>+</sup> kg<sup>-1</sup> (19.2 g kg<sup>-1</sup> KCl). Each diet was prepared by mixing the dry ingredients in a mixer (Hobart, Troy, OH, USA) for 30 min. Subsequently, hot water (~40% by weight) was blended into the mixture until appropriate consistency for pelleting was obtained. The mash was passed through a 3 mm die, and pellets were dried at 40 °C in a forced air kiln to a moisture content of approximately 80 g kg<sup>-1</sup>. Diets were stored at -20 °C until commencement of experimental trials, when they were mechanically crumbled

and sieved to desired size. Each diet was tested in four replicate tanks with 12 juvenile shrimp (mean individual weight 0.5 g) per tank. Shrimp were counted weekly, and ration was calculated assuming a 1.75 feed conversion ratio and a doubling in size until individual shrimp weighed 1 g. Thereafter, a growth rate of 1 g per week was assumed. At the end of a 44-day growth period, shrimp were harvested, counted and group-weighted. Haemolymph osmolality, Cl<sup>-</sup>, K<sup>+</sup> and Na<sup>+</sup> levels were measured using a composite sample from several shrimp per tank. Shrimp and haemolymph samples were then stored at -70 °C pending further analysis.

### Trial 2

The second trial also tested effects of seven practical diets containing 360 g protein kg<sup>-1</sup> and 80 g lipid kg<sup>-1</sup> diet (Table 3), with similar treatment levels as the first experiment. The same purified mineral sources were utilized for NaCl and Mg<sup>2+</sup> as in trial 1, however, a coating agent (CA) (Xtra-Dry; Uniscope Inc., Johnston, CO, USA) was combined at a 50 g CA kg<sup>-1</sup> level (by weight) with each mineral in an attempt to reduce leaching. Furthermore, a chelated amino acid complex (Chelated Minerals Corporation, Salt Lake City, UT, USA) was used as the dietary K<sup>+</sup> source. The following inclusion levels were used replacing cellufil; 10 g NaCl kg<sup>-1</sup> (10.5 g NaCl + 50 g CA kg<sup>-1</sup>); 20 g NaCl kg<sup>-1</sup> (21 g NaCl + 50 g CA kg<sup>-1</sup>), 140 mg kg<sup>-1</sup> Mg<sup>2+</sup> (140 mg kg<sup>-1</sup> from trace mineral premix); 300 mg kg<sup>-1</sup> (1.3 g MgCl<sub>2</sub>·6H<sub>2</sub>O + 5 g CA kg<sup>-1</sup>). Each diet was tested on five replicate tanks with 12 juvenile shrimp (mean individual weight 0.28 g) per tank. Shrimp were counted weekly and the ration was calculated assuming a 1.75 feed conversion ratio and a doubling in size until individual shrimp weighed 1 g. Thereafter, a growth rate of 1 g per week was assumed. At the end of a 49-day growth period, shrimp were harvested, counted and group-weighted. Haemolymph was collected using a syringe, and all samples were pooled in a 1.5 mL Eppendorf tube. The sample was used to determine haemolymph osmolality, chloride, K<sup>+</sup> and Na<sup>+</sup> levels. Shrimp and haemolymph samples were then stored at -70 °C pending further analysis.

### Haemolymph osmotic and ionic concentrations

In order to determine haemolymph osmotic and ionic concentrations, stored samples were thawed on ice and then sonicated (25 W, 30 s, Heat Systems Microson) to disrupt

the clot (Henry *et al.* 2003). Samples were then centrifuged (10 000 *g* for 60 s) to separate the clot from serum. Total osmolality was then determined using a vapour pressure osmometer (Wescor 5100C, Logan, UT, USA). Haemolymph chloride ion concentration was determined by Ag titration (Labconco Chloridometer, Kansas City, MO, USA). Finally, haemolymph Na<sup>+</sup> and K<sup>+</sup> concentrations were determined using flame photometry (Cole Parmer Digital Flame Photometer, Vernon Hills, IL, USA).

### Hepatopancreas mineralization

Whole shrimp were thawed and the hepatopancreas of five intermolt shrimp from each tank were thawed and dissected. Hepatopancreas samples from shrimp in each dietary treatment were pooled into four composite samples from trial 1 and five composite samples from trial 2. Samples were then oven dried and wet ashed as described in Association of Official Analytical Chemists (1984). Subsequently, K<sup>+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup> were analysed by inductively coupled argon plasma spectrophotometry according to standard protocols (Clesceri *et al.* 1998).

### Leaching

Mineral leaching from diets in trial 2 was evaluated in order to assess efficacy of the coating agent used. Two diets (300 Mg kg<sup>-1</sup> Mg<sup>2+</sup> without a coating agent; 20 g NaCl kg<sup>-1</sup> without a coating agent) that were prepared concurrently with diets from trial 2, but not used in the growth study were used as controls. A sample of each of the nine diets was dried overnight at 95 °C. Two subsamples (approximately 5 g each) of each dried diet were placed in 50 mL of distilled H<sub>2</sub>O for 30 min. The water was then passed through a dried and preweighed glass filter and analysed using ICAP (Clesceri *et al.* 1998). The filter was dried to constant weight at 95 °C and weight of leachate determined. The amount of K<sup>+</sup>, Na<sup>+</sup> and Mg<sup>2+</sup> that leached out of each diet was then calculated. There are no published standard methods for determining leaching rates of minerals, hence, we chose to simply place intact feed in distilled water to provide a relative value of what could leach under the most extreme conditions.

### Statistical analysis

Statistical analyses were performed using SAS (version 8.2; SAS Institute, Cary, NC, USA). Data from both experiments were analysed using one-way analysis of variance to deter-

mine if significant differences ( $P \leq 0.05$ ) existed among treatment means. Student–Newman–Keuls multiple comparison test (Steel & Torrie 1980) was used to determine differences among treatment means.

## Results

### Trial 1

There were no significant differences in survival and growth among treatments (Table 4). Survival among treatments ranged from 79% to 92%, being lowest in the 300 Mg kg<sup>-1</sup> Mg<sup>2+</sup> treatment and highest in the 20 g NaCl kg<sup>-1</sup> treatment. Shrimp reared using the basal diet displayed the lowest weight gain (784.7%) while shrimp from the 10 g NaCl kg<sup>-1</sup> (954.3%) and 10 g K<sup>+</sup> kg<sup>-1</sup> (917.1%) treatments had the largest weight gain. Shrimp offered the basal diet exhibited the smallest mean individual weight (4.49 g), although values were not statistically different from other treatments ( $P > 0.05$ ).

### Trial 2

There were no significant differences in per cent weight gain or survival among treatments in trial 2 (Table 4). However,

**Table 4** Weight gain and survival of *L. vannamei* reared in artificial low salinity water fed two experimental diets supplemented with K<sup>+</sup>, Mg<sup>2+</sup> and NaCl

	Mean weight (g)	Weight gain (%)	Survival (%)
Trial 1			
Basal	4.49 ± 0.42 <sup>a</sup>	784 ± 43 <sup>a</sup>	81 ± 4 <sup>a</sup>
10 g NaCl kg <sup>-1</sup>	4.94 ± 0.41 <sup>a</sup>	949 ± 66 <sup>a</sup>	84 ± 9 <sup>a</sup>
20 g NaCl kg <sup>-1</sup>	4.77 ± 0.29 <sup>a</sup>	886 ± 83 <sup>a</sup>	92 ± 6 <sup>a</sup>
150 mg kg <sup>-1</sup>	4.71 ± 0.40 <sup>a</sup>	876 ± 104 <sup>a</sup>	86 ± 14 <sup>a</sup>
300 mg kg <sup>-1</sup>	4.91 ± 0.64 <sup>a</sup>	901 ± 120 <sup>a</sup>	79 ± 10 <sup>a</sup>
5 g K kg <sup>-1</sup>	4.95 ± 0.27 <sup>a</sup>	865 ± 50 <sup>a</sup>	85 ± 4 <sup>a</sup>
10 g K kg <sup>-1</sup>	4.92 ± 0.35 <sup>a</sup>	917 ± 72 <sup>a</sup>	83 ± 6 <sup>a</sup>
PSE <sup>1</sup>	0.206	41	0
Trial 2			
Basal	4.54 ± 0.36 <sup>b</sup>	1515 ± 154 <sup>a</sup>	78 ± 15 <sup>a</sup>
10 g NaCl kg <sup>-1</sup>	4.89 ± 0.34 <sup>ab</sup>	1605 ± 185 <sup>a</sup>	82 ± 9 <sup>a</sup>
20 g NaCl kg <sup>-1</sup>	4.73 ± 0.47 <sup>ab</sup>	1548 ± 198 <sup>a</sup>	75 ± 10 <sup>a</sup>
140 mg kg <sup>-1</sup>	4.53 ± 0.26 <sup>b</sup>	1487 ± 187 <sup>a</sup>	80 ± 11 <sup>a</sup>
300 mg kg <sup>-1</sup>	4.86 ± 0.13 <sup>ab</sup>	1596 ± 59 <sup>a</sup>	80 ± 12 <sup>a</sup>
5 g K kg <sup>-1</sup>	4.91 ± 0.61 <sup>ab</sup>	1617 ± 146 <sup>a</sup>	80 ± 12 <sup>a</sup>
10 g K kg <sup>-1</sup>	5.34 ± 0.27 <sup>a</sup>	1787 ± 163 <sup>a</sup>	78 ± 15 <sup>a</sup>
PSE <sup>1</sup>	0.169	72	0.56

Shrimp from trial 1 had an initial individual weight of 0.5 g, while shrimp from trial 2 had an initial weight of 0.28 g. Values are mean ± standard deviation of four replicates in trial 1 and five replicates in trial 2. Values with different superscript are significantly different ( $P \leq 0.05$ ).

<sup>1</sup> Pooled standard error.

there were significant differences in mean individual weights of shrimp at harvest ( $P = 0.035$ ). The final individual weights of shrimp offered the 10 g K<sup>+</sup> kg<sup>-1</sup> (5.34 g) diet were significantly higher than shrimp offered the basal (4.54 g) and 140 mg kg<sup>-1</sup> Mg<sup>2+</sup> (4.53 g) diets.

#### Haemolymph osmotic and ionic concentrations

There were no significant differences observed among treatments with regards to haemolymph osmolality or chloride concentration in both experimental trials (Table 5). Mean haemolymph osmolalities ( $\pm$  pooled standard error) were  $618 \pm 26$  and  $637 \pm 26$  mmol kg<sup>-1</sup> for trials 1 and 2, respectively. Mean haemolymph chloride concentrations were  $252 \pm 11$  and  $251 \pm 13$  mEq L<sup>-1</sup> for trials 1 and 2, respectively. Finally, mean haemolymph K<sup>+</sup> levels were  $6.67 \pm 0.26$  and  $7.80 \pm 0.37$  mEq L<sup>-1</sup> for trials 1 and 2, respectively, while Na<sup>+</sup> concentrations in both trials 1 and 2 were  $303 \pm 12$  and  $294 \pm 13$  mEq L<sup>-1</sup>, respectively.

#### Hepatopancreas mineralization

There were no significant differences observed in hepatopancreas mineralization among minerals examined (Table 6). In both trials, dietary supplementation of K<sup>+</sup>, Mg<sup>2+</sup> and NaCl did not affect the levels of K<sup>+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup> in the hepatopancreas. The use of a chelated K<sup>+</sup> resulted in

higher hepatopancreas K<sup>+</sup> levels in trial 2 (3.89–4.13 mg g<sup>-1</sup>) compared with trial 1 (2.63–2.79 mg g<sup>-1</sup>) where KCl was utilized as the K<sup>+</sup> source.

#### Leaching

Ions quantified as leachates from diets used in trial 2 were calcium, potassium, magnesium, phosphorous, copper, iron, manganese, sodium and zinc (Table 7). In the 5 and 10 g K<sup>+</sup> kg<sup>-1</sup> treatment, 35.7% and 38.3% of the K<sup>+</sup> leached out of the diet, respectively. In the case of Na<sup>+</sup>, when a coating agent was utilized, approximately 34.5% and 41% of the Na<sup>+</sup> in the feed leached out of the 10 and 20 g NaCl kg<sup>-1</sup> treatments, respectively. In the 20 g NaCl kg<sup>-1</sup> treatment without coating agent, a slightly higher amount of Na<sup>+</sup> leached from the diet (44.3%).

#### Discussion

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 organism in order to ensure proper growth and survival. Traditional practices, such as the application of agricultural fertilizers (K-mag and muriate of potash) directly to the pond water, have been proven effective at improving growth and survival (McNevin *et al.* 2004). However, the use of dietary

	Osmolality (mmol kg <sup>-1</sup> )	Cl (mEq L <sup>-1</sup> )	Na (mEq L <sup>-1</sup> )	K (mEq L <sup>-1</sup> )
Trial 1				
Basal	652 $\pm$ 17	247 $\pm$ 33	310 $\pm$ 12	6.47 $\pm$ 0.38
10 g NaCl kg <sup>-1</sup>	611 $\pm$ 39	248 $\pm$ 16	304 $\pm$ 18	7.10 $\pm$ 0.46
20 g NaCl kg <sup>-1</sup>	633 $\pm$ 53	270 $\pm$ 21	312 $\pm$ 18	6.55 $\pm$ 0.40
150 ppm Mg	595 $\pm$ 33	245 $\pm$ 8	293 $\pm$ 12	7.02 $\pm$ 0.35
300 ppm Mg	630 $\pm$ 58	247 $\pm$ 23	305 $\pm$ 16	6.93 $\pm$ 0.74
5 g K kg <sup>-1</sup>	618 $\pm$ 41	261 $\pm$ 15	300 $\pm$ 12	6.78 $\pm$ 0.46
10 g K kg <sup>-1</sup>	586 $\pm$ 84	243 $\pm$ 23	292 $\pm$ 28	6.34
PSE <sup>1</sup>	25	10	9	0.26
Trial 2				
Basal	632 $\pm$ 43	244 $\pm$ 25	294 $\pm$ 27	7.57 $\pm$ 1.07
10 g NaCl kg <sup>-1</sup>	600 $\pm$ 27	232 $\pm$ 54	283 $\pm$ 23	8.54 $\pm$ 0.93
20 g NaCl kg <sup>-1</sup>	643 $\pm$ 68	253 $\pm$ 27	298 $\pm$ 29	7.70 $\pm$ 0.77
140 ppm Mg	633 $\pm$ 68	249 $\pm$ 13	298 $\pm$ 25	8.12 $\pm$ 0.55
300 ppm Mg	674 $\pm$ 24	279 $\pm$ 15	307 $\pm$ 13	7.64 $\pm$ 0.52
5 g K kg <sup>-1</sup>	648 $\pm$ 57	256 $\pm$ 14	284 $\pm$ 19	7.42 $\pm$ 0.86
10 g K kg <sup>-1</sup>	629 $\pm$ 47	241 $\pm$ 21	293 $\pm$ 28	7.60 $\pm$ 0.49
PSE <sup>1</sup>	26	12	12	0.37

**Table 5** Haemolymph osmolality and ionic concentrations in shrimp fed diets with mineral supplements of K<sup>+</sup>, Mg<sup>2+</sup> and NaCl

Values represent the mean  $\pm$  standard deviation of four replicates in trial 1 and five replicates in trial 2. No significant differences were observed among treatments.

<sup>1</sup> Pooled standard error.

<sup>2</sup> Only one sample analysed due to insufficient haemolymph.

**Table 6** Selected mineral content ( $\text{mg g}^{-1}$ ) of the hepatopancreas for shrimp fed diets with mineral supplements of  $\text{K}^+$ ,  $\text{Mg}^{2+}$  and NaCl

Diets	Na	K	Mg	Ca
<b>Trial 1</b>				
Basal	7.97 ± 1.00	3.45 ± 0.57	0.65 ± 0.11	2.82 ± 0.31
10 g NaCl $\text{kg}^{-1}$	6.91 ± 0.44	2.68 ± 0.16	0.58 ± 0.05	2.49 ± 0.14
20 g NaCl $\text{kg}^{-1}$	7.60 ± 0.25	3.14 ± 0.07	0.63 ± 0.02	3.14 ± 0.30
150 mg $\text{kg}^{-1}$	7.31 ± 0.71	3.18 ± 0.60	0.59 ± 0.10	2.69 ± 1.12
300 mg $\text{kg}^{-1}$	7.04 ± 1.47	3.10 ± 1.00	0.64 ± 0.17	2.67 ± 0.82
5 g K(chelated) $\text{kg}^{-1}$	6.54 ± 0.65	2.62 ± 0.39	0.58 ± 0.11	2.96 ± 1.57
10 g K(chelated) $\text{kg}^{-1}$	6.77 ± 0.76	2.79 ± 0.45	0.55 ± 0.09	2.26 ± 0.49
PSE <sup>1</sup>	0.42	0.27	0.05	0.42
P-value	0.26	0.32	0.81	0.81
<b>Trial 2</b>				
Basal	8.73 ± 2.14	3.74 ± 1.23	0.63 ± 0.15	3.77 ± 0.99
10 g NaCl $\text{kg}^{-1}$	9.52 ± 1.31	3.92 ± 0.77	0.68 ± 0.12	3.34 ± 0.99
20 g NaCl $\text{kg}^{-1}$	9.40 ± 1.18	3.98 ± 0.63	0.62 ± 0.10	3.37 ± 0.96
140 mg $\text{kg}^{-1}$	8.80 ± 1.56	3.78 ± 1.08	0.61 ± 0.12	3.27 ± 2.2
300 mg $\text{kg}^{-1}$	10.04 ± 2.18	4.12 ± 0.67	0.65 ± 0.06	3.83 ± 1.5
5 g K(chelated) $\text{kg}^{-1}$	9.00 ± 1.70	3.88 ± 0.95	0.63 ± 0.12	2.77 ± 0.52
10 g K(chelated) $\text{kg}^{-1}$	9.60 ± 1.72	4.12 ± 0.65	0.66 ± 0.08	3.25 ± 1.1
PSE <sup>1</sup>	0.77	0.40	0.05	0.56
P-value	0.88	0.99	0.96	0.87

Values represent the mean ± standard deviation of four replicates. No significant differences were observed among treatments.

<sup>1</sup> Pooled standard error.

**Table 7** Levels of ions leached ( $\text{g kg}^{-1}$ ) from feed pellets (trial 2) submerged for 30 min in distilled water (DI water)

Diet	Ca	K	Mg	P	Cu	Fe	Mn	Na	Zn
Basal	0.145	3.788	0.288	0.820	0.002	0.008	0.002	0.448	0.016
10 g NaCl $\text{kg}^{-1}$ (C <sup>1</sup> )	0.148	4.163	0.296	0.677	0.002	0.007	0.001	1.833	0.016
20 g NaCl $\text{kg}^{-1}$ (C)	0.163	4.880	0.329	0.701	0.002	0.008	0.002	3.698	0.016
140 mg $\text{kg}^{-1}$	0.141	3.826	0.300	0.801	0.003	0.005	0.001	0.431	0.023
300 mg $\text{kg}^{-1}$ (C)	0.144	4.079	0.316	0.852	0.003	0.007	0.002	0.595	0.017
5 g K $\text{kg}^{-1}$	0.136	6.028	0.334	2.063	0.015	0.009	0.009	0.464	0.015
10 g K $\text{kg}^{-1}$	0.137	8.074	0.354	3.712	0.018	0.009	0.017	0.489	0.014
300 mg $\text{kg}^{-1}$	0.113	3.770	0.291	0.745	0.001	0.006	0.002	0.427	0.015
20 g NaCl $\text{kg}^{-1}$	0.148	5.196	0.341	0.751	0.000	0.007	0.002	3.963	0.016
DI water	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

<sup>1</sup> C = Xtra-Dry coating agent.

supplements may either allow reductions in the level of supplements added to the water or provide additional performance enhancements in these marginal environments. Experiments in the present study were conducted at a salinity of  $4.0 \text{ g L}^{-1}$ , which is comparable with the salinity utilized by commercial shrimp farms in west Alabama. However, as  $\text{K}^+$  concentration in various waters in west Alabama varies by site, consequently, the  $\text{K}^+$  requirement for *L. vannamei* reared in these well waters varies by site. In addition to salinity of the medium, concentrations of individual elements as well as ratios of various ions necessary for normal osmoregulatory function vary from farm to farm (Saoud *et al.* 2003).

Maintenance of potassium balance within the body is necessary for proper functioning of membrane potentials and all life systems. The lack of an adequate supply of  $\text{K}^+$  in the

water profile has been shown to negatively impact survival and growth of *L. vannamei* (McGraw & Scarpa 2003, 2004; Saoud *et al.* 2003). Furthermore, while shrimp reared in full strength seawater and offered a  $\text{K}^+$ -free diet grew normally,  $\text{Mg}^{2+}$  storage in the carapace decreased noticeably (Davis *et al.* 1992). It is apparent that  $\text{K}^+$  levels in the diet affect the physiology of shrimp. In trial 2, shrimp offered the diet containing  $10 \text{ g K}^+ \text{ kg}^{-1}$  amino acid complex yielded significantly greater growth than shrimp fed the basal diet, thus demonstrating benefits of supplementation of chelated  $\text{K}^+$  to diets formulated for shrimp cultured in low salinity waters. Our results are supported by Shiau & Hsieh (2001), who concluded that there is a  $\text{K}^+$  requirement for *Penaeus monodon* that cannot be met solely by the available  $\text{K}^+$  in brackish water containing  $360 \text{ ppm K}^+$ . Additionally, Gong *et al.* (2004) reported increased growth in *L. vannamei* fed a

diet containing KCl, MgO and NaCl compared with a diet without any of these mineral additions at two different low salinity shrimp farms in Arizona. In a field trial we conducted in low salinity waters at a shrimp farm in west Alabama, supplementation of a chelated source of  $K^+$  to the feed increased growth rate of shrimp (unpublished data). Potassium supplementation to feed was also demonstrated to be beneficial to teleosts. Shearer (1988) reported that juvenile chinook salmon, benefitted from dietary supplementation of  $K^+$  when reared in fresh water.

Magnesium is an essential mineral required by crustaceans for normal growth and development (Davis & Lawrence 1997). Magnesium serves as a cofactor in many enzymatic reactions important for normal function, and it is involved in osmoregulation, protein synthesis and growth (Furriel *et al.* 2000). A lack of dietary  $Mg^{2+}$  has been shown to depress  $K^+$  concentrations of the carapace in juvenile *L. vannamei*, indicating a possible interaction between  $K^+$  and  $Mg^{2+}$  (Davis *et al.* 1992). Furthermore, low levels of aqueous  $Mg^{2+}$  have been correlated to reduced survival and growth of postlarval and juvenile shrimp (Saoud *et al.* 2003; Davis *et al.* 2005). Few studies have examined the role of dietary  $Mg^{2+}$  for *L. vannamei* reared in low salinity environments (Gong *et al.* 2004; Cheng *et al.* 2005). Cheng *et al.* (2005) reported no differences in hepatopancreas  $Mg^{2+}$ -ATPase and  $Na^+/K^+$  ATPase activities in diets containing various levels of  $Mg^{2+}$  for *L. vannamei* reared in low salinity waters. These authors also reported a dietary  $Mg^{2+}$  requirement for optimal growth of 2.60–3.46 g  $Mg^{2+}$   $kg^{-1}$  for *L. vannamei* reared in low salinity waters. In the present work, supplementation of  $Mg^{2+}$  did not improve growth and survival of juvenile *L. vannamei* reared in waters deficient in  $Mg^{2+}$ . Differences in results among published reports are probably due to differences in ion profile of culture waters used. Levels of  $Mg^{2+}$  vary considerably depending on the source of the low salinity water (Saoud *et al.* 2003). Thus, the reported requirement of  $Mg^{2+}$  for *L. vannamei* in low salinity waters (Cheng *et al.* 2005) might be beneficial only under the experimental conditions in which their experiment was carried out. Furthermore, supplementation of  $Mg^{2+}$  might affect  $K^+$  metabolism (see Davis & Lawrence 1997) thus masking any beneficial attributes of  $Mg^{2+}$  supplementation. The use of a chelated  $Mg^{2+}$  source might improve delivery of this divalent cation to the animal and thus should be examined in the future.

Dietary supplementation of NaCl has the potential to provide benefits for euryhaline species. 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 NaCl (Holsapple 1990; Gatlin *et al.* 1992). Gatlin *et al.* (1992) observed that in juvenile red drum ion losses at low salinity can significantly impair growth, however, this limitation was overcome by dietary supplementation of NaCl. Shrimp in the present experiment that were offered a feed supplemented with NaCl appeared to survive better than in treatments without NaCl supplementation although differences were not statistically significant. Sodium and chloride ions are the major osmolytes in the haemolymph of crustaceans, including shrimp (Castille & Lawrence 1981; Mantel & Farmer 1983; Pequeux 1995). Dietary supplementation of these essential osmolytes potentially counteracts losses to the medium that cannot be counteracted by gill uptake in very low salinity environments. The importance of NaCl regulation was demonstrated by McFarland & Lee (1963) who found that in low salinity environments *L. setiferus* maintains relatively high levels of serum sodium and chloride. However, there is a point beyond which organisms cannot osmoregulate efficiently and thus become stressed. A dietary supplementation of salt was shown to remedy such stress in some cases. Gatlin *et al.* (1992) concluded that extra salt supplemented to diets of juvenile red drum compensated for salt deficiency observed in fish reared in dilute media. However, the benefits of NaCl supplementation are not universal for euryhaline species, as several studies have also documented the lack of beneficial effects on growth, feed efficiency and feed intake such as in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) (Shaw *et al.* 1975; MacLeod 1978). Dietary supplementation of NaCl in feed for shrimp reared in low salinity environments appears promising and merits further examination.

Haemolymph osmolality was not affected by dietary treatment. This is logical, as osmolality is usually a function of the salinity of the medium (Iwata & Shigueno 1980; Gong *et al.* 2004) and not its ion profile. *Litopenaeus vannamei* tend to be very euryhaline and have been shown to live in salinities ranging from 1 g  $L^{-1}$  to more than 40 g  $L^{-1}$  (Bray *et al.* 1994), which indicates that they are excellent osmoregulators as long as ion ratios in the water are adequate. Such is not the case in inland low salinity well waters, and that explains increased mortality and decreased growth even when measures of haemolymph osmolality were within adequate ranges. Gong *et al.* (2004) noted that shrimp reared with feed containing dietary sources of KCl, magnesium oxide, NaCl, phospholipids, and cholesterol had a more robust osmoregulatory capacity and a more stable osmolality compared with shrimp fed a diet containing no supplements. Their results may have been due to feed supplements other than the

minerals, but they did not report effects of individual supplements. Levels of  $\text{Cl}^-$ ,  $\text{K}^+$  and  $\text{Na}^+$  obtained in the present study were similar to those observed in other penaeids reared in low salinity water (McFarland & Lee 1963; Dall & Smith 1981). Supplementation of these minerals to the diet did not appear to affect levels in the haemolymph. This can be attributed to several factors such as leaching of minerals before consumption, sloppy feeding typical of shrimp and insufficient osmoregulatory capacity. Further studies where mineral levels in the water are modified concurrently with dietary supplements have to be performed before definitive conclusions can be made.

Levels of  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  in the hepatopancreas were not affected by dietary supplementation of  $\text{K}^+$ ,  $\text{Mg}^{2+}$  or  $\text{NaCl}$  in both growth trials (Table 6). When  $\text{K}^+$  levels in the hepatopancreas were compared among all shrimp offered diet 1 and diet 2, they were found to be higher in the second trial (3.75–4.13  $\text{mg g}^{-1}$ ) when compared with the first trial (2.63–3.45  $\text{mg g}^{-1}$ ). The observed results are probably due to the use of a coating agent for  $\text{Mg}^{2+}$  and  $\text{NaCl}$  diets, and the use of a chelated  $\text{K}^+$  in the diets supplemented with  $\text{K}^+$  in trial 2. Additionally, it is noteworthy that although  $\text{Ca}^{2+}$  was not supplemented in excess of the dietary requirement in the present study, hepatopancreas levels of  $\text{Ca}^{2+}$  were higher in trial 2 where a coating agent was utilized than in trial 1 where no coating agent was added to the minerals (Table 6). Further growth trials utilizing diets containing a mineral coating agent in conjunction with diets receiving no coating agent might prove informative in terms of their application to feeds utilized for the culture of *L. vannamei* in low salinity environments. As no differences in hepatopancreas storage of  $\text{K}^+$ ,  $\text{Mg}^{2+}$  or  $\text{Na}^+$  were observed in the present experiment it is probable that levels of these ions in the rearing medium and diet were sufficient to carry out normal physiological processes. This is further supported by the lack of differences between the measured osmolytes ( $\text{K}^+$  and  $\text{Na}^+$ ) in the haemolymph. If levels of these ions were near threshold lethal limits one would expect significant reductions in both haemolymph osmolytes and hepatopancreas storage which could translate to reduced growth and survival.

In studies examining the effectiveness of dietary mineral supplementation, the amount of minerals that remain available to the organism after the feed is submerged in water is seldom examined. In the first growth trial there were no visible effects of the various minerals on growth. Thus the question of availability of the minerals arose. In our experiment, approximately 45% (5  $\text{g kg}^{-1}$  supplement) and 43% (10  $\text{g kg}^{-1}$  supplement) of the  $\text{K}^+$  in the chelated  $\text{K}^+$  amino acid complex leached from the feed after 30 min of sub-

mersion, suggesting that a substantial amount of  $\text{K}^+$  still remains in the feed and is available to the shrimp. Approximately 34.5%, 41% and 44.5% of the  $\text{Na}^+$  leached out of the 10  $\text{g NaCl kg}^{-1}$  diet with the CA, 20  $\text{g NaCl kg}^{-1}$  diet with CA, and the 20  $\text{g NaCl kg}^{-1}$  diet without the CA, respectively. When comparing the 20  $\text{g NaCl kg}^{-1}$  diets with and without the coating agent, the diet with the coating agent lost a slightly smaller amount of  $\text{Na}^+$ . Very little  $\text{Mg}^{2+}$  leached out of either the CA-coated or non-CA-coated feed. Based on these results the CA was not effective in laboratory prepared feed and the use of a chelated form of  $\text{Mg}^{2+}$  is worth examining. Information concerning the bioavailability of chelated minerals versus chloride salts in feed is limited for crustaceans species in the literature. However, there are a number of studies examining this issue with fish species (Paripatananont & Lovell 1997; Apines-Amar *et al.* 2004; Sá *et al.* 2005; Sarker *et al.* 2005). Paripatananont & Lovell (1997) determined that net absorption of chelated minerals (copper, iron, manganese, selenium and zinc proteinates) in channel catfish (*Ictalurus punctatus*) were significantly higher (39.3% in purified diets and 81.1% in practical diets) when compared with inorganic forms (copper sulphate pentahydrate, ferrous sulphate heptahydrate, manganese sulphate monohydrate, sodium selenite and zinc sulphate heptahydrate). Apines-Amar *et al.* (2004) reported that the dietary absorption of zinc and manganese in the form of amino acid chelates were higher than their inorganic salt counterparts in rainbow trout (*Oncorhynchus mykiss*). Sarker *et al.* (2005) found that chelated amino acid trace elements improved fish growth, feed conversion ratio and nutrient retention in red sea bream (*Pagrus major*). Our study demonstrated an increase in growth in shrimp fed a diet containing a 10 g potassium amino acid complex  $\text{kg}^{-1}$  diet in a low salinity (4  $\text{g L}^{-1}$ ) laboratory setting. Further studies in a pond production setting are necessary to determine if these same trends will be confirmed in the field.

## Conclusion

Dietary supplementation of minerals essential for osmoregulatory processes appears to be a promising practice for enhancing growth and survival of *L. vannamei* cultured in low salinity waters. The results of our study suggest that there are beneficial effects on growth of *L. vannamei* reared in 4.0  $\text{g L}^{-1}$  inland low salinity well-water when the feed is supplemented with 10  $\text{g K}^+ \text{kg}^{-1}$  diet. Although there were no significant differences observed with  $\text{Mg}^{2+}$  and  $\text{NaCl}$  supplementation, there was an observable trend of increasing growth that should be investigated more closely. However, these preliminary

results do not yet justify a recommendation that mineral supplements in feed can completely replace mineral supplements to the culture medium. The degree to which specific osmolytes are regulated over a range of low salinity waters with non-oceanic ion ratio profiles needs to be studied. We believe that a balance between feed supplements and partial remediation of water composition coupled with low discharge at harvest will be the optimal method to farm shrimp in inland low salinity well-water farms in west Alabama.

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