

Supplementation of Chelated Magnesium to Diets of the Pacific White Shrimp, *Litopenaeus vannamei*, Reared in Low-salinity Waters of West Alabama

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Abstract

Shrimp farmers using inland low-salinity waters in west Alabama have traditionally used agricultural fertilizers (K-Mag®, muriate of potash) to raise pond water levels of potassium (K) and magnesium (Mg) to improve the rearing medium for *Litopenaeus vannamei*. Laboratory and farm trials were performed to investigate the potential of using dietary supplementation of Mg instead of costly agricultural fertilizers. A 5-wk laboratory trial was devised to test four diets with varying levels of Mg supplementation (0, 0.15, 0.30, and 0.60% Mg chelate) using a magnesium chelate (MgC)-amino acid complex. Juvenile shrimp were stocked into artificial low-salinity water (5 ppt) designed to contain low levels of Mg. A farm trial was also conducted to test the same diets under field conditions. Although both laboratory and on-farm trials revealed a trend for increased growth using the diet with the highest Mg supplement, results were not significantly different. The use of magnesium chelates as dietary supplements at levels higher than the requirement level to enhance survival, growth, and osmoregulatory capacity of shrimp reared in inland low-salinity well waters appeared to have limited potential. Until effective specialized diet formulations are produced, farmers should continue to supplement pond waters with fertilizers containing K and Mg.

Farmers of the Pacific white shrimp, *Litopenaeus vannamei*, using inland low-salinity well waters (LSWWs) are faced with the challenge of rearing animals in less than ideal environments. Depending on their source, inland waters available to culture shrimp can possess variable ionic compositions (K, Mg, and Ca levels) and salinity (Boyd and Thunjai 2003; Saoud et al. 2003). Despite the ability of *L. vannamei* to tolerate a wide range of environmental salinities, farmers still have to address problems associated with variation of ionic profiles among pond waters (Saoud et al. 2003) that can lead to poor growth and survival of shrimp (Davis et al. 2005; Roy et al. 2007a).

Low-salinity problems can generally be solved through addition of specific ions (e.g., K and Mg) to culture water (McGraw and Scarpa 2003; Roy et al. 2007a). Davis et al. (2005) reported that

under controlled laboratory conditions, K deficiencies of the water are remediable through potassium chloride supplementation. Commercial farmers using inland LSWW are mitigating this problem by increasing levels of K and Mg in their pond waters through addition of muriate of potash and/or K-Mag® (McNevin et al. 2004). Unfortunately, adding large amounts of agricultural fertilizers to ponds can be costly. Treated water may be discarded during harvest or lost in overflow during the rainy season. Furthermore, ions can be complexed with the pond soil, thus reducing bioavailability to shrimp (Boyd 2007).

For good culture conditions, the ionic profile of the low-salinity water must have appropriate levels and ratios of specific ions (Na : K, Mg : Ca, etc.), which should be similar to that of full-strength seawater that, for example, would be 28:1 for the Na : K ratio (Roy et al. 2007a). It has been reported that low-salinity water deficient in Mg resulted in higher respiration rates

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and reduced survival in juvenile shrimp (Roy et al. 2007a). Zhu et al. (2004) reported that a Na : K ratio of greater than 43:1 resulted in decreased activity and even death of shrimp reared at 30 ppt. Deficiencies in the ionic profile of water used for shrimp culture have typically been remediated by supplementing K and Mg directly to pond water (McNevin et al. 2004). Farmers in west Alabama try to maintain the Na : K ratios in their pond waters at less than 40:1. However, although the supplementation of minerals to pond waters does work, from a financial point of view, it can be costly. This is particularly true when multiple applications are necessary because certain soils adsorb ions from the water.

An alternative approach is to counteract low Na : K and Mg : Ca ratios in the water by supplementing K and Mg to the feed, thus allowing shrimp to absorb these ions from the digestive tract. Because of the success associated with mineral supplementation to low-salinity pond waters, the potential of dietary supplementation of minerals merits further investigation. Supplementation of K and Mg in excess of the dietary requirement has been proposed as a means to improve the osmoregulatory capacity of shrimp, thus increasing growth and survival (Gong et al. 2004; Cheng et al. 2005; Saoud and Davis 2005; Roy et al. 2006, 2007b; Saoud et al. 2007). Cheng et al. (2005) reported a dietary Mg (2.6–3.46 g Mg/kg) requirement of *L. vannamei* for optimal growth in low-salinity water. However, because levels of Mg vary considerably depending on the source of low-salinity water (Saoud et al. 2003), the requirement reported by Cheng et al. (2005) is probably only adequate under the low-salinity conditions in which their experiment was carried out. Roy et al. (2007b) demonstrated a significant increase in growth of shrimp associated with supplementation of a chelated K–amino acid complex. They reported that chloride salts (potassium chloride, magnesium chloride, and sodium chloride) did not prove effective in improving shrimp growth. Another study examining the supplementation of chelated K to diets of shrimp reared in low-salinity water originating from production ponds at two west Alabama farms demonstrated no significant differences in growth, survival,

weight gain, or osmoregulatory capacity of *L. vannamei* (Saoud et al. 2007).

The present study was performed to evaluate the effects of supplementation of Mg chelated to an amino acid complex in practical diets of *L. vannamei* reared in Mg-deficient inland LSWW.

Materials and Methods

The present work was conducted using reconstituted low-salinity water at the E.W. Shell Fisheries Center at Auburn University in Auburn, Alabama, and at a low-salinity shrimp farm in west Alabama. Four experimental diets were formulated to contain 36% protein and 8% lipid (Table 1). A magnesium chelate (MgC) was added to three of the diets at levels of 0.15, 0.30, and 0.60% using a magnesium–amino acid chelated complex that contained 10% Mg by weight (Chelated Minerals Corporation, Salt Lake City, UT, USA). Each diet was prepared by mixing the dry ingredients in a mixer (Hobart Model A-200ft, Hobart Corp., Troy, OH, USA) for 30 min. Hot water, at approximately 40% by weight, was blended in 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 8% and stored at –20 C. Diets were analyzed for Mg content by a commercial laboratory (New Jersey Feed Lab Inc., Trenton, NJ, USA) at the end of the trial (Diet 1: 0.164%, Diet 2: 0.181%, Diet 3: 0.195%, and Diet 4: 0.222%).

Laboratory Trial

Artificial low-salinity water was prepared 3 wk prior to the commencement of the experiment using well water from the E.W. Shell Fisheries Center mixed with an artificial sea salt. The well water (0.1 ppt salinity) at the E.W. Shell Fisheries Center has 2.9 mg/L K, 6.0 mg/L Mg, 30.1 mg/L Ca, and 6.5 mg/L Na. Twelve 150-L polyethylene tanks connected to a common biofilter were filled with well water and 0.5 ppt salinity reconstituted seawater (Crystal Sea® /Forty Fathoms® Marinemix, Marine Enterprises International, Baltimore, MD, USA) supplemented with 40 mg/L calcium from

TABLE 1. *Ingredient composition of diets for laboratory and farm trials (g/100 g dry weight) supplemented with a magnesium chelate (MgC).^a*

Ingredient	Diet 1 (basal)	Diet 2 (0.15% MgC)	Diet 3 (0.30% MgC)	Diet 4 (0.60% MgC)
Fish meal ^b	3.00	3.00	3.00	3.00
Poultry meal ^c	15.30	15.30	15.30	15.30
Soybean meal ^d	33.60	33.60	33.60	33.60
Menhaden fish oil ^e	4.52	4.52	4.52	4.52
Wheat starch ^f	10.78	10.63	12.98	15.18
Whole wheat ^f	19.60	19.60	19.60	19.60
Trace mineral premix ^g	0.50	0.50	0.50	0.50
Vitamin premix ^h	1.80	1.80	1.80	1.80
Stay C ⁱ	0.10	0.10	0.10	0.10
Calcium phosphate dibasic ^f	0.80	0.80	0.80	0.80
Cellulose ^j	5.00	5.00	2.50	0.00
Lecithin ^k	0.50	0.50	0.50	0.50
Cholesterol ^k	0.20	0.20	0.20	0.20
Gelatin ^l	4.00	4.00	4.00	4.00
MgC	0.00	0.15	0.30	0.60

^a Diets were formulated to contain 35.2% protein and 8% lipid. Diets were analyzed for Mg content by New Jersey Feed Lab, Inc., Trenton, New Jersey, USA (Diet 1: 0.164%, Diet 2: 0.181%, Diet 3: 0.195%, and Diet 4: 0.222%).

^b Special SelectTM, Omega Protein Inc., Hammond, Louisiana, USA.

^c Griffin Industries, Inc., Cold Springs, Kentucky, USA.

^d Dehulled solvent-extracted soybean meal, Southern States Cooperative Inc., Richmond, Virginia, USA.

^e Omega Protein Inc., Reedville, Virginia, USA.

^f MP Biochemicals Inc., Aurora, Ohio, USA.

^g g/100 g premix: cobalt chloride, 0.004; cupric sulfate pentahydrate, 0.250; ferrous sulfate heptahydrate, 4.0; magnesium sulfate heptahydrate, 28.398; manganous sulfate monohydrate, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate heptahydrate, 13.193; and filler, 53.428.

^h g/kg 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 B12, 0.002; choline chloride, 100.0; inositol, 5.0; menadione, 2.0; vitamin A acetate (20,000 IU/g), 5.0; vitamin D3 (400,000 IU/g), 0.002; DL-alpha-tocopheryl acetate (250 IU/g), 8.0; and alpha-cellulose, 865.266.

ⁱ 250 mg/kg active C supplied by Stay C[®] (L-ascorbyl-2-polyphosphate, 25% vitamin C activity), Roche Vitamins Inc., Parsippany, New Jersey, USA.

^j ICN, Aurora, Ohio, USA.

^k Fisher Scientific, Pittsburgh, Pennsylvania, USA.

^l Chelated Minerals Corporation, Salt Lake City, Utah, USA.

CaCl₂·2H₂O and 35 mg/L K from KCl. Water salinity was increased to 5.0 ppt using rock salt (NaCl). Mineral composition of the resultant

water was similar to that of inland LSWW found in west Alabama. Twelve juvenile *L. vannamei* (0.22 g initial weight) were stocked into each tank of the recirculating system. Daily feed ration, including the initial daily ration, was calculated based on expected growth assuming a feed conversion ratio of 1.75 and a doubling in size (approximately every 7 d) until the estimated shrimp weights were in excess of 1 g. Thereafter, a growth rate of 1 g/wk was assumed. Shrimp were fed four times daily using automatic feeders. Five weeks after the start of the experiment, the shrimp in each replicate tank were harvested, counted, and group weighed by replicate tank. Dissolved oxygen (6.6 ± 0.2 mg/L), temperature (28.7 ± 1.1 C), pH (8.5 ± 0.1), and salinity (5.0 ± 0.1 ppt) were measured daily and remained within acceptable limits. Total ammonia-nitrogen (0.15 ± 0.14 mg/L) and nitrite-nitrogen (0.05 ± 0.21 mg/L) were measured biweekly and also remained within acceptable limits for the culture of *L. vannamei*. Total ammonia-nitrogen was analyzed according to Solorzano (1969), and nitrite-nitrogen was measured according to Parsons et al. (1985).

Farm Trial

A series of circular tanks (0.8 m² bottom surface area and 600 L water volume) were used for the farm tank study, and a flow-through recirculating system was built adjacent to a pond. Water from the ponds was continuously pumped into the tanks, and the overflow drained back into the pond via a central standpipe. Water was aerated using submersible diffusers (two per tank) connected to an air blower. Twenty juvenile *L. vannamei* (1.50 g initial weight) were stocked into each of the 16 tanks. Each diet was offered to four replicate tanks. Daily feed ration was calculated similarly to the first experiment. Nine weeks after the start of the experiment, all shrimp were harvested, counted, and group weighed by replicate tank. Water samples from the supply pond were taken at the start and at the end of the experiment for determination of ion profile (aqueous levels of K, Mg, Ca, Na, and other ions) and osmolality analysis (Table 2). Furthermore, hemolymph samples were taken from all shrimp and pooled by tank to measure osmolality.

In order to evaluate the relationship between dietary Mg intake and hemolymph ion levels, sub-samples of hemolymph were also taken for ion analyses. Dissolved oxygen (7.5 ± 0.9 mg/L), temperature (31.6 ± 2.0 C), pH (9.4 ± 0.5), and salinity (2.5 ± 0.1 ppt) were monitored daily and remained within acceptable ranges for the culture of *L. vannamei*. Ammonia-nitrogen (0.7 ± 0.69 mg/L) and nitrite-nitrogen (0.35 ± 0.45 mg/L) were measured once weekly.

Osmolality and Ion Analysis

Pond water and hemolymph osmolality were evaluated using a vapor pressure osmometer (Wescor Vapro, Logan, UT, USA) and reported as mmol/kg. Hemolymph was withdrawn from the pericardial cavity using a 25-gauge needle and 1-cc syringe inserted beneath the carapace at the cephalothorax–abdominal junction. Hemolymph samples were withdrawn from all shrimp in the experiment (one composite sample per tank) and stored at -20 C. Prior to analyses, hemolymph samples were thawed on ice and then sonicated (Omni Ruptor 250, OMNI International, Marietta, GA, USA). The samples were then centrifuged at 10,000 *g* for 60 sec to separate the clot from serum. Total osmolality was then measured using 10 μ L of serum by dew point depression. Levels of K, Mg, Ca, and Na (ionic profile) in the water and hemolymph were determined using inductively coupled argon plasma (ICAP) spectrophotometry by the Soil Testing Laboratory at Auburn University and reported as meq/L (Clesceri et al. 1998).

TABLE 2. Ionic composition (mg/L) of low-salinity waters used to culture *Litopenaeus vannamei* in laboratory and farm trials as compared to seawater.

	Laboratory trial	Farm trial	Seawater ^a
Minerals (mg/L)			
Sodium	1531	677	10,500
Potassium	40.2	33	380
Magnesium	62.5	16	1350
Calcium	165.9	19	400
Salinity (ppt)	5.0	2.5	34.5
Ratios			
Na : K	38.1	20.5	28.3
Ca : K	4.1	0.6	1.1
Mg : Ca	0.4	0.8	3.4

^a Goldberg (1963).

Whole-Body Mineral Content

Samples of dried whole shrimp tissue from the farm trial were acid digested and analyzed by the Soil Testing Laboratory at Auburn University (Auburn, AL, USA) for mineral content using ICAP spectrophotometry (Donohue and Aho 1992). Four shrimp were randomly selected from each tank and stored at -20 C. Prior to analysis, whole shrimp were thawed and pooled as one composite sample per tank.

Statistical Analysis

Statistical analyses were performed using SAS (version 8.2; SAS Institute, Cary, NC, USA). All data were analyzed using one-way ANOVA and Student–Newman–Keuls multiple range test to determine if significant differences ($P \leq 0.05$) existed among treatment means (Steel and Torrie 1980).

Results and Discussion

Rearing shrimp using low-salinity ground water presents a unique opportunity to expand the culture of marine species away from coastal areas. Unfortunately, it also has a number of challenges, especially when dealing with water with ionic profiles different from that of dilute seawater. Saoud et al. (2003) reported that low-salinity shrimp farms in west Alabama had less than optimal ionic profiles for raising *L. vannamei* because they were deficient in K and Mg. Consequently, farmers must enhance the K and Mg levels of the water to maximize growth and survival of shrimp during the production cycle. Because of the expense of these amendments, farmers are interested in using dietary supplements as a possible alternative or mechanism to further stabilize production results. Results from both the laboratory and the farm trial (Tables 3 and 4) revealed no significant differences ($P > 0.05$) in survival, final weight, weight gain, or hemolymph osmolality in shrimp among dietary treatments. Survival and growth at both sites were good and demonstrate the ability of shrimp to tolerate a wide range of salinities and ionic profiles. In both trials, shrimp offered the diet containing the highest level of Mg from MgC demonstrated

TABLE 3. Final weight (g), survival (%), weight gain (%), and hemolymph osmolality (mmol/kg) for *Litopenaeus vannamei* offered diets with varying levels of magnesium chelate (MgC) in low-salinity water during the laboratory trial.^a

Laboratory trial	Initial weight (g)	Final weight (g)	Survival (%)	Weight gain (%)	Osmolality (mmol/kg)
Basal	0.22	2.7 ± 0.12	75.6 ± 7.8	1131.6 ± 142.5	737.3 ± 56.7
0.15% MgC	0.22	3.0 ± 0.02	60.0 ± 6.7	1267.4 ± 75.5	707.7 ± 28.3
0.30% MgC	0.22	2.7 ± 0.26	75.6 ± 10.2	1145.6 ± 76.4	680.7 ± 29.9
0.60% MgC	0.22	3.1 ± 0.26	64.4 ± 10.2	1289.6 ± 141.7	721.3 ± 66.7
PSE		0.10	4.4	57.0	24.2
P value		0.11	0.14	0.28	0.56

PSE = pooled standard error.

^a Values represent the mean of four replicates. No significant differences were observed ($P > 0.05$) among treatment means.

TABLE 4. Final weight (g), survival (%), weight gain (%), and hemolymph osmolality (mmol/kg) for *Litopenaeus vannamei* offered diets with varying levels of magnesium chelate (MgC) in low-salinity water during the farm trial.^a

Farm trial	Initial weight (g)	Final weight (g)	Survival (%)	Weight gain (%)	Osmolality (mmol/kg)
Basal	1.50	15.9 ± 1.1	90.0 ± 7.1	956.6 ± 58.9	687.0 ± 6.5
0.15% MgC	1.50	16.2 ± 0.6	92.5 ± 6.5	981.4 ± 65.8	711.5 ± 23.5
0.30% MgC	1.50	15.5 ± 1.8	92.5 ± 2.9	938.9 ± 106.5	710.5 ± 52.1
0.60% MgC	1.50	16.8 ± 1.0	91.3 ± 7.5	1016.7 ± 54.4	734.0 ± 39.2
PSE		0.60	3.1	37.2	18.4
P value		0.50	0.93	0.51	0.39

PSE = pooled standard error.

^a Values represent the mean of four replicates. No significant differences were observed ($P > 0.05$).

numerically higher weight gain, although results were not statistically significant ($P > 0.05$). The basal diet used in this experiment contained 0.164% Mg and would be considered replete for Mg (Davis et al. 1992; Davis and Gatlin 1996; Cheng et al. 2005).

Hemolymph Ca, K, Mg, and Na of shrimp from the farm trial varied only slightly in all treatments, displaying no significant differences (Table 5). Hemolymph K ranged between 8.99 and 9.37 meq/L across all treatments examined. Hemolymph Ca (9.89–10.52 meq/L), Mg (1.74–1.87 meq/L), and Na (258.6–269.0 meq/L) were also maintained by shrimp within narrow ranges and do not seem to be influenced by dietary supplementation of Mg in excess of the requirement. While in some cases authors have reported that dietary mineral supplementation can affect hemolymph osmolality of shrimp (Gong et al. 2004), in our study, dietary supplementation of Mg did not alter hemolymph ion levels. These patterns are similar to those observed in previous studies (Roy et al. 2007a, 2007b) conducted in low-salinity waters of west Alabama. While the shrimp offered the 0.60 and 0.30% MgC diet

had slightly lower hemolymph Mg (1.74–1.75 meq/L) than those offered the basal and 0.15% MgC diet (1.85–1.87 meq/L), these differences were not statistically significant. Supplementation of Mg in excess of the dietary requirement does not appear to provide any additional osmoregulatory benefit to shrimp reared in low-salinity waters. It is also interesting to note that no differences in whole-body mineral content were observed in the farm trial (Table 6).

TABLE 5. Hemolymph ion levels (meq/L) for *Litopenaeus vannamei* offered diets containing varying levels of Mg from magnesium chelate (MgC) during the farm trial.^a

Diet	Ca (meq/L)	K (meq/L)	Mg (meq/L)	Na (meq/L)
Basal	10.52 ± 0.41	9.37 ± 1.44	1.85 ± 0.19	258.6 ± 8.3
0.15% MgC	10.11 ± 1.56	9.37 ± 1.36	1.87 ± 0.18	265.6 ± 11.9
0.30% MgC	9.93 ± 1.52	8.99 ± 0.91	1.75 ± 0.35	258.8 ± 13.2
0.60% MgC	9.89 ± 1.86	9.24 ± 0.82	1.74 ± 0.16	269.0 ± 9.0
PSE	0.73	0.58	0.12	5.4
P value	0.92	0.96	0.79	0.47

PSE = pooled standard error.

^a No significant differences were observed ($P > 0.05$).

TABLE 6. Selected mineral content of whole shrimp (*Litopenaeus vannamei*) from the farm trial.^a

Diet	Ca (g/100 g)	K (g/100 g)	Mg (g/100 g)	Na (mg/kg)	P (g/100 g)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)
Basal	2.85 ± 0.28	1.12 ± 0.08	0.19 ± 0.012	5664 ± 718	1.21 ± 0.08	35.2 ± 3.4	30.6 ± 20.3	22.1 ± 5.2	51.5 ± 3.1
0.15% MgC	2.67 ± 0.11	1.09 ± 0.06	0.19 ± 0.010	5287 ± 689	1.19 ± 0.03	35.8 ± 5.0	37.6 ± 16.6	20.4 ± 4.5	50.1 ± 1.7
0.30% MgC	2.66 ± 0.21	1.09 ± 0.06	0.19 ± 0.005	5350 ± 47	1.21 ± 0.03	33.1 ± 2.0	24.4 ± 6.80	16.3 ± 6.6	49.9 ± 2.0
0.60% MgC	2.79 ± 0.28	1.12 ± 0.03	0.19 ± 0.009	5753 ± 579	1.20 ± 0.03	41.7 ± 4.8	40.6 ± 19.4	23.8 ± 3.5	53.9 ± 3.2
P value	0.63	0.80	0.78	0.66	0.95	0.052	0.21	0.24	0.16
PSE	0.12	0.030	0.005	311	0.023	2.0	8.33	2.54	1.29

PSE = pooled standard error.

^a No significant differences were observed ($P \geq 0.05$).

While incrementally higher levels of Mg were offered to shrimp, there were no significant changes in the storage of Mg in the hepatopancreas, further confirming that the diets were replete and there was no added benefit to enhanced Mg levels of the diet.

Results from the present study indicate that dietary supplementation of Mg from MgC, in excess of the Mg requirement for shrimp, did not provide any additional benefits (growth, tissue mineralization, or hemolymph ion levels) to shrimp reared in low-salinity water containing an adequate ionic profile (i.e., appropriate Na : K and Mg : Ca ratios). These results are in agreement with those from other studies (Roy et al. 2007b; Saoud et al. 2007), which suggest that dietary supplementation of minerals (either as chelates or as chloride salts) confers only marginal benefits when compared with supplementation of fertilizers (K-Mag®, muriate of potash) rich in K and Mg directly to the water. Minerals supplemented individually or in combination (i.e., multiple minerals supplemented to the same diet) have not proven successful in west Alabama farm trials where natural productivity plays a role (Saoud et al. 2007). The lack of an optimal suite of ions in the culture water cannot be offset solely by dietary supplementation of minerals. Better survival and growth are achieved when minerals are supplemented directly to the water, albeit the supplementation of Mg to the feed would have been a more cost-effective solution.

In summary, the enhancement of dietary Mg levels above optimum dietary requirements resulted in minimal shift in weight gain of the

shrimp and does not appear to protect the shrimp from odd ionic profiles of the water. Hence, farmers would benefit more by supplementing minerals directly to their culture water rather than adding minerals in excess of dietary requirements to the feed.

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