

Effects of lecithin and cholesterol supplementation to practical diets for *Litopenaeus vannamei* reared in low salinity waters

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Abstract

The culture of shrimp in inland low salinity waters is a developing industry in many regions of the world, including west Alabama. These inland low salinity waters are often deficient in key ions necessary for normal physiological function. In west Alabama, farmers normally remedy ionic deficiencies in the water profile through the addition of fertilizers containing K^+ and Mg^{2+} . It has been suggested that increasing phospholipids (lecithin) and cholesterol in excess of dietary requirement improve osmoregulatory capacity in *Litopenaeus vannamei*, thus leading to better survival and growth under low salinity conditions. Cholesterol is an essential sterol involved in the molting process in shrimp. Phospholipids are important in cholesterol transport, facilitate the storage of lipids in the hepatopancreas, an important energy reserve during the molting process and are an important component of cell membranes. In order to investigate the possibility of improving growth and survival under stressful (i.e. low K^+ and Mg^{2+}) rearing conditions, a series of lab and on-farm experiments were conducted. Two separate 35 day laboratory studies were conducted in reconstituted low salinity (4.0 ppt, low K^+) waters. In both trials, five practical diets were formulated to contain 36% protein and 8% lipid, and supplemented with varying levels of cholesterol and lecithin. Three of these diets were utilized for an additional experiment carried out on-site at two different low salinity shrimp farms in west Alabama. Results from the lab trials indicated no significant differences in survival, growth, or percent weight gain among treatments. Survival, final weight, and percent weight gain ranged from 68% to 77%, 2.70–3.0 g, 415–471% in experiment 1, and 56–69%, 2.7–3.2 g, 1572–1913% in experiment 2. These results indicate that the shrimp were stressed in both experiments, and there were no apparent benefits to supplementing lecithin and cholesterol in excess of the dietary requirement. Two on farm trials were conducted in parallel using either a mediated water source (Farm 1) to produce low stress or waters. At farm 1, survival, final weight, percent weight gain, and FCR ranged from 93.8% to 98.8%, 4.48–5.23 g, 4273–4901%, and 1.79–2.06, respectively. At farm 2 shrimp had lower survival (37.5–47.5%), lower final weight (2.65–3.25 g), lower percent weight gain (2342–3088%), and higher FCRs (6.85–10.64). No benefits from lecithin and cholesterol supplementation in excess of the dietary requirement were observed when compared to the basal diet under any test conditions. Based on results of the present study, dietary supplementation of cholesterol and phospholipids in excess of the requirement is not warranted for *L. vannamei* reared in low salinity waters.

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1. Introduction

The inland culture of shrimp, particularly the Pacific white shrimp, *Litopenaeus vannamei*, is currently being

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undertaken in west Alabama using inland low salinity well waters (LSWW). Depending on their source, LSWW can be of varying salinities, and therefore possess different ionic compositions (Boyd and Thunjai, 2003). Despite the success by some farmers in culturing *L. vannamei* in LSWW, problems still arise due to mineral deficiencies in the ionic profiles of pond waters (Saoud et al., 2003; Atwood et al., 2003). The lack of a necessary mix of ions essential for osmoregulation (Castille and Lawrence, 1981; Pequeux, 1995), such as potassium (K^+) and (Mg^{2+}) has been shown to limit growth and survival of shrimp (Saoud et al., 2003; Davis et al., 2005). Farmers in west Alabama have improved growth and survival of *L. vannamei* in low salinity waters by raising the K^+ and Mg^{2+} levels of their pond waters (McNevin et al., 2004), yet there are still indications or incidences in which the shrimp appear to be stressed.

In a study conducted in Arizona, Gong et al. (2004) suggested incorporating phospholipids (lecithin) and cholesterol in excess of the dietary requirement as a potential means of improving osmoregulatory capacity in *L. vannamei*, thus leading to better survival and growth under low salinity conditions. Gong et al. (2004) observed increased osmoregulatory capacity of shrimp reared in LSWW through dietary addition of K^+ , Mg^{2+} , NaCl, lecithin, and cholesterol. However, the influence of each supplement was not individually evaluated. Cholesterol is an essential sterol involved in the molting process in shrimp (Teshima, 1972) and is important in growth and survival of crustaceans. Phospholipids are important in cholesterol transport, facilitate the storage of lipids in the hepatopancreas, which serves as an important energy reserve during the molting process, and are an important component of cell membranes (Clarke and Wickins, 1980; Teshima et al., 1986). Since shrimp are unable to synthesize cholesterol de novo or synthesize phospholipids in sufficient quantities to meet their dietary requirements, these ingredients are considered essential nutrients for shrimp (Gong et al., 2000).

Mortalities that occur at farms utilizing LSWW during the production period are believed to be associated with the diminished ability of juvenile and subadult shrimp to hyper-osmoregulate in low salinity waters (Saoud et al., 2003; Gong et al., 2004). The inability to effectively maintain adequate hemolymph mineral balance can result in molt-associated mortality (Gong et al., 2004). Gong et al. (2004) also reported low levels of lipid in the hepatopancreas of shrimp reared in low salinity waters, which is a major energy reserve utilized by shrimp during molting (Clarke and Wickins, 1980; Gong et al., 2000).

Dietary supplementation of phospholipids and cholesterol could potentially improve growth and survival of *L. vannamei* raised in low salinity waters. Moreover, such supplementation could prove a more cost-effective strategy when compared to adding large amounts of agricultural fertilizers to increase the concentrations of desired ions in ponds at commercial shrimp farms using inland low salinity waters (McNevin et al., 2004). The objective of the present study was to evaluate claims that phospholipid and cholesterol supplementation above the dietary requirement could improve growth and survival of *L. vannamei* in low salinity waters.

2. Materials and methods

2.1. Indoor laboratory trials

Laboratory experiments were conducted at the North Auburn Fisheries Research Station in Auburn, Alabama. Post-larval *L. vannamei* for experiment 1 were obtained from GMSB Shrimp Hatchery (Summerland Key, FL, USA), while shrimp utilized in experiment 2 were obtained from Harlingen shrimp farm (Bayview, TX, USA). Post-larvae were acclimated down to low salinity water (4.0 ppt) over a period of 8 h and maintained in a 220 L polyethylene nursery tank connected to a biological filter. During the first week PLs were offered a combination of *Artemia* nauplii (200 nauplii per shrimp) and a commercial feed, PL Redi-Reserve (Ziegler Bros. Gardner, Pennsylvania) at 25–50% body weight. Thereafter, shrimp were fed a commercial feed (Rangen 35% protein, Buhl, Idaho) and reared in the nursery system until they were of appropriate size for commencement of growth trials. Both experiments were conducted in a 2400 L recirculating system, containing a series of 60 L aquaria. Artificial low salinity water was prepared two weeks prior to the commencement of each experiment by adding 0.5 ppt reconstituted seawater (Crystal Sea Salt, Baltimore, Maryland) and a supplement of calcium ($CaCl_2 \cdot 2H_2O$). Salinity was then raised to 4.0 ppt using agricultural grade NaCl. Levels of K^+ in the experimental water were below optimal levels for the culture of *L. vannamei* in low salinity water. Light regime was set at 16 h day and 8 h night using fluorescent bulbs. Dissolved oxygen (DO), pH, salinity, and temperature were measured daily, whereas ammonia and nitrites were measured twice weekly using methods described by Solorzano (1969) and Parsons et al. (1985), respectively. For lab trial 1 dissolved oxygen ($7.27 \pm 0.34 \text{ mg L}^{-1}$), temperature ($28.6 \pm 1.0 \text{ }^\circ\text{C}$), pH (8.1 ± 0.1), salinity ($4.1 \pm 0.04 \text{ g L}^{-1}$), ammonia ($0.03 \pm 0.02 \text{ mg L}^{-1}$), and nitrites ($0.14 \pm 0.18 \text{ mg L}^{-1}$) remained within acceptable limits

for the culture of this species. Likewise, for lab trial 2 dissolved oxygen ($6.83 \pm 0.44 \text{ mg L}^{-1}$), temperature ($28.9 \pm 1.3 \text{ }^\circ\text{C}$), pH (8.0 ± 0.1), ammonia ($0.05 \pm 0.12 \text{ mg L}^{-1}$), and nitrites ($0.04 \pm 0.03 \text{ mg L}^{-1}$) remained within acceptable culture requirements. The experimental water was analyzed for major ions by inductively coupled argon plasma spectrophotometry according to standard protocols (Clesceri et al., 1998).

The diets were formulated to contain 35% protein and 8% lipid. Treatments consisted of four diets with varying levels of dietary lecithin and cholesterol (Table 1) and a fifth diet containing no lecithin or cholesterol supplementation. The cholesterol content of the basal diet which did not receive cholesterol supplementation (diet 5) was verified to contain 0.08% cholesterol. Diets were prepared by mixing the ingredients in a mixer (Hobart, Troy, Ohio) for 30 min. Subsequently, hot water was added to the mixture until appropriate consistency for pelleting was obtained. Diets were then passed through a meat grinder and a 3 mm die. Pellets were air dried ($<50 \text{ }^\circ\text{C}$) to a moisture content of less than 10%. Lab trial 2 was a repeat of lab trial 1 using the same diets and an additional commercial diet (Rangen 35, 0) as a commercial reference.

In lab trial 1, 20 experimental tanks (5 treatments, 4 replicates) were each stocked with 12 juvenile shrimp (mean individual weight 0.529 g). In lab trial 2, 30 experimental tanks (6 treatments, 5 replicates) were each stocked with 12 juvenile shrimp (mean initial weight 0.1 g). In both trials, 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 one gram. Thereafter, a growth rate of one gram per week was assumed. Feed inputs were adjusted for mortalities on a weekly basis. At the end of a 35 day growth period, shrimp were harvested, counted and group weighed.

2.2. Farm trials

Three of the experimental diets (diets 1, 2, 3) were utilized for an additional experiment carried out at two low salinity shrimp farms in west Alabama. Flow-through systems consisting of twelve, 600 L tanks (3 dietary treatments, 4 replicates) were set up at these two farms containing waters with different ionic profiles. Twenty shrimp (0.10 g initial weight) were stocked per tank and maintained for six weeks. One farm had K^+ supplemented to the low salinity water (1.5 ppt) and is

Table 1

Composition (g/100 g dry weight) of practical diets designed to contain 35% protein and 8% lipid that were used in the growth trials

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal ^a	3.00	3.00	3.00	3.00	3.00
Poultry meal ^b	15.30	15.30	15.30	15.30	15.30
Soybean meal ^c	33.60	33.60	33.60	33.60	33.60
Menhaden fish oil ^d	4.52	4.52	4.52	4.52	4.52
Wheat starch ^e	9.15	8.95	9.70	8.58	9.98
Whole wheat ^e	19.60	19.60	19.60	19.60	19.60
Trace mineral premix ^f	0.50	0.50	0.50	0.50	0.50
Vitamin premix ^g	1.80	1.80	1.80	1.80	1.80
Stay C ^h	0.10	0.10	0.10	0.10	0.10
Calcium phosphate ^c	2.40	2.40	2.40	2.40	2.40
Cellulfil ⁱ	5.00	5.00	3.15	4.87	4.87
Lecithin ^j	0.50	0.50	1.00	1.00	0.00
Cholesterol ^j	0.20	0.40	0.20	0.40	0.00
Gelatin ⁱ	4.00	4.00	4.00	4.00	4.00

^a Special Select™, Omega Protein Inc., Hammond, Louisiana, USA.

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

^c De-hulled solvent extracted soybean meal, Southern Sates Cooperative Inc., Richmond Virginia, USA.

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

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

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

^g 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-tocopherol acetate (250 IU/g) 8.0, Alpha-cellulose 865.266.

^h 250 mg/kg active C supplied by Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), Hoffman-La Roche Vitamins Inc., Parsippany, New Jersey, USA.

ⁱ ICN, Aurora, Ohio, USA.

^j Fisher Scientific, Pittsburgh, Pennsylvania, USA.

Table 2

Response of *L. vannamei* reared in artificial low salinity waters in separate laboratory trials (initial weight trial 1:0.52 g; initial weight trial 2:0.1 g) fed experimental diets supplemented with different levels of lecithin and cholesterol

Lab trial 1	Supplement		Final indiv. wt. (g)	Percent wt. gain (%)	Survival (%)
	Chol.	Lec.			
Diet 1	0.2	0.5	2.94±0.24	461.9±62.9	73.4±12.5
Diet 2	0.4	0.5	2.70±0.18	416.2±55.4	75.0±5.7
Diet 3	0.2	1.0	2.86±0.30	440.8±48.1	76.8±10.8
Diet 4	0.4	1.0	3.03±0.24	471.4±56.6	68.4±7.1
Diet 5	0.0	0.0	2.80±0.23	415.3±42.6	71.6±15.3
PSE *			0.108	24.0	0.049
P-value			0.28	0.36	0.78
Lab trial 2					
Diet 1	0.2	0.5	2.91±0.26	1814.0±212.5	63.4±16.1
Diet 2	0.4	0.5	2.96±0.35	1805.7±227.2	58.4±11.8
Diet 3	0.2	1.0	3.05±0.11	1887.3±93.8	65.0±14.8
Diet 4	0.4	1.0	3.20±0.36	1912.9±215.8	58.4±13.1
Diet 5	0.0	0.0	2.68±0.42	1572.8±364.1	56.6±7.1
Commercial reference diet *	0.0	0.0	2.79±0.40	1663.1±317.3	68.8±14.1
PSE **			0.15	114.2	0.060
P-value			0.21	0.71	0.28

Values represent means±standard deviation.

* 35% Protein, Rangen 35,0 (Buhl, Idaho).

** Pooled Standard Error.

considered a low stress environment, while water at the other farm (3.5 ppt) did not receive K⁺ supplementation and is considered a high stress environment. Shrimp were fed assuming a 1.75 feed conversion ratio and a doubling in size until individual shrimp weighed one gram. Thereafter, a growth rate of one gram per week was assumed. At the end of a 42 day growth period, shrimp were harvested, counted and group weighed. Percent survival and FCR were also assessed. Experimental waters were analyzed for major ions by inductively coupled argon plasma spectrophotometry according to standard protocols (Clesceri et al., 1998). Throughout the experiment, dissolved oxygen (DO), pH, salinity, and temperature were measured daily, whereas ammonia (Solorzano, 1969) was measured weekly. At Farm 1 dissolved oxygen (10.40±3.9 mg L⁻¹), temperature (29.6±2.1 °C), pH (8.7±0.3), salinity (1.4±0.11 g L⁻¹), and ammonia (0.72±0.23 mg L⁻¹) remained within acceptable limits for the culture of *L. vannamei*. At Farm 2, dissolved oxygen (10.58±3.7 mg L⁻¹), temperature (28.9±3.0 °C), pH (8.2±0.2), salinity (3.0±0.15 g L⁻¹) ammonia (0.64±0.47 mg L⁻¹), also remained within acceptable limits.

2.3. Statistical analysis

Statistical analyses were performed using SAS (version 9.2, SAS Institute, Cary, North Carolina). Data

from both experiments were analyzed using one-way analysis of variance to determine if significant differences ($P \leq 0.05$) existed among treatment means. Student–Newman–Keuls multiple comparison test (Steel and Torrie, 1980) was utilized to determine differences among treatment means.

Table 3

Ionic composition (mg L⁻¹) of low salinity waters used to culture *L. vannamei* at North Auburn Research Unit compared to seawater

Minerals (mg L ⁻¹)	Laboratory trial 1	Laboratory trial 2	Farm 1	Farm 2	Seawater *
Sodium	1415	1763	367.4	1187.5	10500
Potassium	7.7	7.6	8.3	7.5	380
Magnesium	25.3	25.2	4.6	13.1	1350
Calcium	70.2	93.2	21.8	56.2	400
Phosphorous	1.2	5.6	0.1	<0.1	–
Zinc	<0.1	<0.1	<0.1	<0.1	0.005–0.014
Iron	<0.1	<0.1	<0.1	<0.1	0.002–0.02
Copper	<0.1	<0.1	<0.1	<0.1	0.001–0.09
Manganese	<0.1	<0.1	<0.1	<0.1	0.001
Salinity(ppt)	4.0	4.0	1.4	3.0	34.5
Ratios					
Na:K	183.8:1	232:1	44.3:1	158.3:1	28:1
Ca:K	9.1:1	7.3:1	2.6:1	7.5:1	1:0.95
Mg:Ca	0.36:1	0.27:1	0.21:1	0.23:1	3.4:1

* (Goldberg, 1963).

3. Results

3.1. Indoor laboratory trials

In trial 1 there were no significant differences in survival, mean individual weight, and percent weight gain among treatments (Table 2), although growth trends in the data were apparent. Mean individual weight ranged from 2.70 to 3.03 g. Survivals in this trial ranged from 68.4% to 76.8% with diet 4 and diet 3 resulting in lowest and highest survivals, respectively. Shrimp reared using the diet containing no lecithin and cholesterol supplementation (diet 5) displayed the least amount of percent weight gain (415.3%) while shrimp offered the diet containing the highest amount of lecithin and cholesterol supplementation (diet 4) displayed the highest percent weight gain (471.4%). Trial 2 yielded similar results to Trial 1, with no significant differences in mean individual weight, percent weight gain, or survival among treatments. Mean individual weights ranged from 2.68 to 3.20 g for shrimp offered the commercial 35% protein shrimp feed as a control. The diet containing no lecithin and cholesterol supplementation (diet 5) yielded the lowest mean individual weights and percent weight gains. Survivals were slightly lower than in trial 1, ranging from 58.4% to 68.8%.

3.2. Farm trials

Overall, shrimp performed better at the low stress farm in which the water received K^+ supplementation but there was no additional benefit incurred due to dietary lecithin and cholesterol supplementation above

dietary requirement. Results from the on-site trials at farm 1 (low stress environment) revealed no significant differences in mean final individual weight (4.48–4.77 g), percent weight gain (4272.8–4639.7%), and survival (93.4–97.5%), or FCR (1.99–2.06) among treatments (Table 4). The on-site trial conducted at farm 2 (high stress environment) which receives no K^+ supplementation to the water also revealed no significant differences in mean final individual weight (2.65–3.25), percent weight gain (2342.2–3087.7%), and survival (37.5–43.8%) or FCR (7.26–10.64).

4. Discussion

Farmers that utilize inland LSWW for commercial production of shrimp may not have optimal water available for maximum growth and survival when compared to coastal waters (Saoud et al., 2003). In addition to having low salinities, ionic profiles of LSWW sources are often deficient in key ions essential for osmotic and ionic regulation. Thus, mineral amendments in the form of fertilizers rich in K^+ and Mg^{2+} , dietary supplementation of minerals essential for osmoregulation (K^+ , Mg^{2+} , and $NaCl$), dietary supplementation of amino acids, and dietary supplementation of phospholipids and cholesterol have all been suggested as potential avenues whereby the osmoregulatory capacity of shrimp cultured in low salinity waters might be improved (Gong et al., 2004; McGraw and Scarpa, 2003; McNevin et al., 2004; Saoud and Davis, 2005; Roy et al., unpublished data). In theory, improved osmoregulatory capacity would result in less expenditure of energy directed towards regulation of hemolymph osmolality, therefore better survival and growth.

Reported cholesterol requirements for *L. vannamei* range from 0.23% to 0.42% for *L. vannamei* reared in outdoor tanks (Duerr and Walsh, 1996) to 0.5% for postlarval *L. vannamei* (Emery, 1987). Gong et al. (2000) reported that cholesterol requirement for *L. vannamei* in the absence of supplemental phospholipids was 0.35%. However, supplementation of 1.5% and 3% phospholipid reduced dietary cholesterol requirement to 0.14% and 0.13%, respectively (Gong et al., 2000). In the present study increasing the dietary supplement of cholesterol and lecithin from 0.2% to 0.4% and 0.5–1.0% did not improve growth or survival of *L. vannamei* in either laboratory or farm trials. In another study conducted in low salinity waters Gong et al. (2004) supplemented 0.1% cholesterol and 1.5% lecithin to an experimental diet also supplemented with 0.5% potassium chloride, 0.8% magnesium oxide, and 0.5% sodium chloride. In addition, different diets were

Table 4
Response of *L. vannamei* reared in artificial low salinity waters in separate farm trials fed experimental diets supplemented with different levels of lecithin and cholesterol

Farm 1 (low stress)	Final indiv. wt. (g)	Percent wt. gain (%)	Survival (%)	FCR
Diet 1 (basal)	4.74	4639.7	93.4	2.06
Diet 2	4.77	4660.5	95.0	1.99
Diet 3	4.48	4272.8	97.5	2.03
PSE*	0.246	265.2	4.23	0.130
<i>P</i> -value	0.67	0.53	0.82	0.94
Farm 2 (high stress)				
Diet 1 (basal)	3.25	3087.7	41.3	7.26
Diet 2	2.65	2342.2	37.5	10.64
Diet 3	2.77	2508.4	43.8	8.38
PSE*	0.24	274.1	4.89	0.970
<i>P</i> -value	0.23	0.19	0.67	0.091

* Pooled Standard Error.

utilized on different farms, further confounding their results. They compared their diet to a control diet without supplementation of any minerals or additional cholesterol and lecithin. Gong et al. (2004) concluded that the experimental diet resulted in improved osmoregulatory capacity and larger shrimp at harvest. However, it is unclear which supplemented ingredient, combination of ingredients, or site specific conditions (i.e. different farm locations) were responsible for the observed effects. Based on our results from both lab and farm trials, dietary supplementation of phospholipids and cholesterol in excess of requirement did not provide any advantages in terms of survival and growth of *L. vannamei* reared in low salinity waters.

In the present work, K^+ levels in reconstituted low salinity waters were intentionally kept low (high Na:K ratio) to stress the shrimp and observe whether dietary lecithin and cholesterol in excess of requirement would provide an additional advantage in the absence of adequate K^+ . The unsuitable water composition (inadequate Na:K ratio) in which the shrimp were reared was most likely responsible for the poor growth and survival observed in both laboratory trials and farm trial 2. The Na:K ratio in natural seawater is approximately 28:1, and inadequate Na:K ratios have been attributed to poor growth and survival of both marine fish and crustaceans reared in saline water. Zhu et al. (2004) observed poor survival at high Na:K ratios (187.3:1) in *L. vannamei* reared at 30 ppt. Optimal Na:K ratio at 30 ppt ranged between 40 and 43:1, while suboptimal ratios resulted in additional energetic costs for the shrimp (Zhu et al., 2004). Forsberg et al. (1996) reported that survival in red drum cultured in inland saline water was correlated to Na:K and Cl:K ratios. Na:K ratios in lab trial 1, lab trial 2, and farm trial 2 ranged from 158:1 to 232:1. At farm 1, where best growth and survival at the lowest rearing salinity (1.4 ppt) were observed, the Na:K ratio (approximately 44:1) was closer to what is found in natural seawater (Tables 3 and 4). However, as in the laboratory trials, no additional advantage was observed in shrimp offered diets supplemented with lecithin and cholesterol in excess of dietary requirement.

5. Conclusion

Results from the present study confirm that growth and survival of juvenile shrimp are suppressed in LSWW with inadequate Na:K ratios. Irregardless of the Na:K ratio, dietary supplementation of phospholipid and cholesterol in excess of the dietary requirement did not improve growth or survival and is not

warranted. Based on current information, farmers with inadequate levels of K^+ in their water should continue to supplement their pond waters with agricultural fertilizers containing sources of both potassium and magnesium. Further studies are necessary to evaluate the effects of other nutritional supplements, such as minerals and non-essential free amino acids, that could improve growth and survival of *L. vannamei* reared in inland LSWW.

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