North American Journal of Aquaculture 69:265–270, 2007

DOI: 10.1577/A06-045.1

[Article]

Chelated Potassium and Arginine Supplementation in Diets of Pacific White Shrimp Reared in Low-Salinity Waters of West Alabama

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Abstract.—Dietary supplements have been proposed as a potential remediation strategy to counteract mineral deficiencies in low-salinity well waters (LSWW) used for shrimp culture in Alabama. Existing strategies (i.e., application of fertilizers such as K-mag and muriate of potash) are costly to farmers attempting to raise levels of potassium (K^+) in their ponds. Previous laboratory studies using dietary supplements of chelated K^+ increased growth but not survival of Pacific white shrimp *Litopenaeus vannamei* reared in low-salinity water. To determine whether this approach is applicable in the field, two growth trials were conducted in flow-through outdoor tanks that used water from farm ponds. The first trial (6 weeks) was conducted simultaneously at two separate low-salinity farms in west Alabama and evaluated two practical diets, one with a chelated K^+ supplement and one without. One of these farms supplements K^+ to its water and is considered a low-stress environment (LSE), while the other farm does not supplement its water and is considered a high-stress environment (HSE). Results indicated that dietary supplementation of K^+ (1.0%) in the absence of an appropriate ionic profile (i.e., the HSE) failed to enhance growth or survival of Pacific white shrimp. Another 9-week growth trial was conducted at the LSE farm. Shrimp were offered either a practical basal diet or the basal diet supplemented with 0.5% chelated K^+, 1.0% chelated K^+, or 0.4% arginine. Although no statistically significant effects of chelated K^+ supplementation were observed, results of this experiment and two other experiments showed an increase in growth associated with chelated K^+ supplementation, indicating a marginal benefit. Present results do not justify K^+ supplementation of diets for Pacific white shrimp reared in inland LSWW. Better shrimp survival and growth are observed when K^+ is added to culture waters to mitigate imbalances in Na:K and Cl:K ratios.

Inland shrimp farming in low-salinity well waters (LSWW) is a growing industry in the United States and various other countries around the world (Boyd and Thunjai 2003; Saoud et al. 2003; McNevin et al. 2004). Inland aquaculture of marine species is desirable for many reasons, notably biosecurity, reduced property prices, and availability of saline waters that are unsuitable for traditional agriculture (Hopkins et al. 1996). However, previous research showed that inland well waters were generally very low in salinity and had ionic compositions that were significantly different from those of seawater (Boyd and Thunjai 2003; Saoud et al. 2003). Moreover, potassium (K^+) was deficient in the majority of these waters (Saoud et al. 2003; McGraw and Scarpa 2003; McNevin et al. 2004).

Low-salinity problems can be solved in many cases through addition of specific ions to culture water. Under controlled laboratory settings, K^+ deficiencies of the water were shown to be remediable through the addition of KCl to culture waters (Davis et al. 2005). Commercial aquaculturists using inland LSWW are mitigating the problem by increasing the K^+ levels in pond waters through addition of muriate of potash, K-Mag®, or both (McNevin et al. 2004). Unfortunately, adding large amounts of salt or K^+ compounds to ponds is costly, especially since treated water may be discarded during harvest or lost in overflow during the rainy season. Nutritional supplements that improve the osmoregulatory capacity of shrimp have been proposed as another method of mitigating low-salinity water (Gong et al. 2004; Roy et al. 2006). Arginine could be one such supplement since it is easily phosphorylated as a high-energy derivative controlling cell content of ATP (Schoffeniels 1970), which is necessary for driving the Na^+–K^+ ATPase pump during osmoregulation (Lucu and Towle 2003). Gong et al. (2004) and Saoud and Davis (2005) proposed dietary supplementation of K^+ and energy compounds to remedy low concentrations in culture waters. Addition of K^+ in the form of chelated K^+ to diets of shrimp reared in low-salinity waters in the laboratory improved growth (Roy et al. 2007). In this experiment, we evaluated the effects of supplementation with arginine and K^+ (chelated to an amino acid complex) in practical diets

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Received June 21, 2006; accepted December 8, 2006

TABLE 1.—Ingredient composition of Pacific white shrimp diets used in Alabama farm trials (g/100 g dry weight of feed). Diets were formulated to contain 35.2% protein and 8% lipid and were supplemented with chelated K+ or arginine.

Ingredient	Diet 1 (basal)	Diet 2 0.5% K	Diet 3 1% K	Diet 4 Arginine
Fish meal[a]	3.00	3.00	3.00	3.00
Poultry meal[b]	15.30	15.30	15.30	15.30
Soybean meal[c]	33.60	33.60	33.60	33.60
Menhaden fish oil[d]	4.52	4.52	4.52	4.52
Wheat starch[e]	9.15	6.65	5.20	8.87
Whole wheat[e]	19.60	19.60	19.60	19.60
Trace mineral premix[f]	0.50	0.50	0.50	0.50
Vitamin premix[g]	1.80	1.80	1.80	1.80
Stay-C[h]	0.10	0.10	0.10	0.10
Calcium phosphate[e]	2.40	2.40	2.40	2.40
Cellufil[i]	5.00	5.00	3.95	4.87
Lecithin[j]	0.50	0.50	0.50	0.50
Cholesterol[j]	0.20	0.20	0.20	0.20
Gelatin[i]	4.00	4.00	4.00	4.00
Chelated K^+[k]	0.00	2.50	5.00	0.00
L-Arginine[i]	0.00	0.00	0.00	0.41

[a] Special Select, Omega Protein, Inc., Hammond, Louisiana.

[b] Griffin Industries, Inc., Cold Springs, Kentucky.

[c] Dehulled solvent extracted soybean meal, Southern Sates Cooperative, Inc., Richmond Virginia.

[d] Omega Protein, Inc., Reedville, Virginia.

[e] MP Biochemicals, Inc., Aurora, Ohio.

[f] Premix (g/100 g): cobalt chloride 0.004, cupric sulfate pentahydrate 0.250, ferrous sulfate 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, filler 53.428.

[g] Premix (g/kg): thiamin HCl 0.5, riboflavin 3.0, pyrodoxine 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, alpha-cellulose 865.266.

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

[i] ICN, Aurora, Ohio.

[j] Fisher Scientific, Pittsburgh, Pennsylvania.

[k] Chelated Minerals Corporation, Salt Lake City, Utah.

of Pacific white shrimp *Litopenaeus vannamei* reared outdoors in LSWW.

Methods

This work was conducted in 2005 and 2006 at two low-salinity farms in west Alabama that use a LSWW source to fill their ponds. One farm remedies ionic deficiency in pond water through supplementation of muriate of potash and is considered a low-stress environment (LSE), whereas the second farm does not treat pond water and is considered a high-stress environment (HSE). A series of circular tanks (bottom surface area = 0.8 m^2; water volume = 600 L) were set up adjacent to a pond at each of the farms. Water from the ponds was continuously pumped into the tanks, and the overflow drained back to the ponds via a central standpipe. Water was aerated using submersible diffusers (two per tank) connected to a regenerative blower. Water for the HSE had a salinity of 3.0‰ and a K^+ concentration of 7.5 mg/L (Na:K ratio = 158.3:1). Water for the LSE had a salinity of 1.4‰ and a K^+ concentration of 8.3 mg/L (Na:K ratio = 44.3:1). Four experimental diets were prepared at the Aquatic Animal Nutrition Laboratory at Auburn University, Auburn, Alabama. Diets were formulated to contain 35.2% protein and 8% lipid (Table 1). Potassium was added to two of the diets at a level of 0.5% and 1.0% by weight using a K^+–amino acid chelated complex that contained 20% K by weight (Chelated Minerals Corporation, Salt Lake City, Utah). An additional diet was supplemented with 0.4% arginine.

Trial 1.—Pacific white shrimp juveniles (initial weight = 0.10 g) were stocked into eight tanks (20 shrimp/tank) at each of the two farms on the same day. Shrimp in four tanks at each farm were offered the basal diet, while shrimp in the other four tanks were offered the diet supplemented with 1% K^+. Ration was calculated based on an assumed feed conversion ratio (FCR) of 1.75 and an assumed doubling in size (approximately every 3–4 d) until individual shrimp weighed 1 g. Thereafter, a growth rate of 1 g/week was assumed. Six weeks after the start of the experiment, the shrimp were harvested, counted, and weighed as a group. At the LSE farm, dissolved oxygen (mean ± SE

= 10.40 ± 3.9 mg/L), temperature (29.6 ± 2.1°C), pH (8.7 ± 0.3), salinity (1.4 ± 0.11‰), and ammonia (0.72 ± 0.23 mg/L) remained within acceptable limits for the culture of Pacific white shrimp. At the HSE farm, dissolved oxygen (10.58 ± 3.7 mg/L), temperature (28.9 ± 3.0°C), pH (8.2 ± 0.2), salinity (3.0 ± 0.15‰), and ammonia (0.64 ± 0.47 mg/L) also remained within acceptable limits.

Trial 2.—The second experiment was performed solely at the LSE farm using the previously described system. The experiment was performed to replicate results of trial 1, to evaluate two levels of K^+ supplementation and determine whether dietary arginine supplementation would improve survival and growth of shrimp reared in LSWW. Twenty juvenile Pacific white shrimp (initial weight = 0.34 g) were stocked into each of 16 tanks. Each of the four experimental diets was offered to shrimp in four randomly selected tanks. Ration was calculated similarly to the first experiment. Nine weeks after the start of the experiment, all shrimp were harvested, counted, and group weighed. A water sample from the supply pond was taken at the start and end of the experiment for ion profile and osmolality analysis. Furthermore, hemolymph samples were taken from all shrimp and samples from each tank were pooled for osmolality and K^+ concentration analysis. Dissolved oxygen (mean ± SE = 7.24 ± 1.16 mg/L), temperature (30.7 ± 2.42°C), pH (9.7 ± 2.8), and salinity (1.3 ± 0.03‰) were monitored daily and remained within acceptable ranges for Pacific white shrimp culture. Ammonia nitrogen (0.75 ± 0.32 mg/L) was measured once weekly and also remained within acceptable limits.

Osmolality and K^+ analysis.—Pond water and hemolymph osmolality was evaluated using a vapor pressure osmometer (Wescor, Logan, Utah; 5100C) and was reported in units of micromoles per kilogram. Water ion profile was determined using inductively coupled argon plasma (ICAP) spectrophotometry (Clesceri et al. 1998), and K^+ levels in hemolymph and water were determined using a digital flame photometer (Cole Parmer, Vernon Hills, Illinois; Model 2655–00).

Whole-body mineral content..—In trial 1, samples of dried whole shrimp tissue were acid digested and analyzed by the Soil Testing Laboratory at Auburn University for mineral content using ICAP spectrophotometry (Donohue and Aho 1992).

Statistical analysis.—Statistical analyses were performed using the Statistical Analysis System (SAS version 8.2; SAS Institute, Cary, North Carolina). Data from the first experiment were analyzed by one-way and two-way analysis of variance (ANOVA). In the second experiment, survival, growth, and hemolymph K^+ concentration data were analyzed using one-way ANOVA. To determine whether significant differences ($P \leq 0.05$) existed among treatment means, the Student–Newman–Keuls multiple-range test was used (Steel and Torrie 1980).

TABLE 2:—Ionic composition (mg/L) of low-salinity waters used to culture Pacific white shrimp at two Alabama farm sites (one low-stress environment [LSE] and one high-stress environment [HSE]) as compared with seawater.

Mineral	LSE	HSE	Seawater[a]
Na	367.4	1,187.5	10,500
K	8.3	7.5	380
Mg	4.6	13.1	1,350
Ca	21.8	56.2	400
P	0.1	<0.1	—
Zn	<0.1	<0.1	0.005–0.014
Fe	<0.1	<0.1	0.002–0.02
Cu	<0.1	<0.1	0.001–0.09
Mn	<0.1	<0.1	0.001
Salinity (‰)	1.4	3.0	34.5
Na:K ratio	44.3	158.3	28

[a] From Goldberg (1963).

Results and Discussion

Water composition at both farms differed from that of seawater (Table 2), and the differences were probably responsible for the observed differences in growth and survival of shrimp. There was no effect of dietary K^+ supplementation on shrimp survival or growth (Table 3). These results confirm an effect of the ionic profile of culture water. It does not appear that this effect can be corrected solely through dietary supplements. The fact that salinity was higher at the HSE farm (3.0‰) than at the LSE farm (1.4‰) suggests that salinity did not affect shrimp growth and survival. Pond water in both farms had similar concentrations of K^+ (7.5 and 8.3 mg/L); however, the HSE had a higher Na:K ratio (158:1) than the LSE (44:1), which presumably affected shrimp health (Table 2). Although various authors have discussed the effects of K^+ concentration on shrimp well-being (see McGraw and Scarpa 2003; Saoud et al. 2003), they have often overlooked or failed to stress that ionic imbalances in culture waters (e.g., K^+ to salinity ratios) are more important than K^+ concentration per se. Boyd and Thunjai (2003) showed that K^+ concentrations were generally much lower in inland LSWW than in diluted seawater with similar salinity; they suggested that K^+ addition to culture waters improved production. Zhu et al. (2004) found that different Na:K molar ratios in artificial seawater significantly affected growth, feed intake, and energy allocation of Pacific white shrimp juveniles. Zhu et al. (2004) also stated that the best

TABLE 3.—Mean initial weight, final mean weight, percent survival, and feed conversion ratio (FCR = feed offered/biomass harvested) of Pacific white shrimp postlarvae reared for 6 weeks at two Alabama farms and offered feeds with (W) and without (WO) supplementation of an amino acid–K complex. Data were analyzed by two-way ANOVA.

Farm site or statistic	Feed treatment or factor	Initial weight (g)	Final weight (g)	Survival (%)	FCR
High-stress environment	W	2.1	2.98	48	6.9
High-stress environment	WO	2.1	3.25	41	7.3
Low-stress environment	W	2.1	5.11	99	1.8
Low-stress environment	WO	2.1	4.74	94	2.1
		***P*-values**			
	Feed		0.85	0.10	0.47
	Farm		0.0001	0.0001	0.0001
	Feed × Farm		0.21	0.85	0.88

Na:K molar ratio for growth of juvenile Pacific white shrimp was between 40:1 and 43:1, while ratios higher than 150:1 were detrimental to shrimp health. Their results corroborate our findings that the Na:K ratio for HSE water was 158.3:1, while the ratio for LSE water was 44.3:1. Furthermore, in previous studies conducted in our laboratory, Roy (2006) observed reduced survival and growth of Pacific white shrimp reared in waters containing an Na:K ratio of 119:1 relative to those of shrimp reared in waters containing 68:1, 48:1, and 29:1 ratios.

The low K^+ concentration relative to Cl^- and Na^+ concentrations in pond water would cause an imbalance in the level of K^+ on the basolateral side of gill Cl cells, thus increasing activity of Na^+–K^+ ATPase to restore electrochemical stasis. The imbalance would also disrupt the Na–K–2Cl co-transporter, which would affect Na^+ and Cl^- secretions. To maintain equilibrium, K^+ levels on the basolateral side of cells must be replenished from intracellular K^+, which depends on K^+ diffusion from the water as well as nutritive uptake to maintain adequate concentrations. The disruption of the Na^+:K^+ or Cl^-:K^+ ratio ultimately affects hemolymph K^+ levels. Low levels of K^+ in the hemolymph subsequently affect neuromuscular function (Robertson 1960). Such a phenomenon could explain lethargy and abdominal cramping observed in shrimp maintained in low-K^+ waters. When K^+ is added to these waters, symptoms of stress are dramatically reduced within days (authors' personal observations on LSWW shrimp farms). However, levels of K^+ added to feeds are not of sufficient quantity to mitigate low concentrations in the water and regulate Na:K ratios. Results of trial 1 show that irrespective of K^+ levels in the water, whole-body K^+ levels of Pacific white shrimp are strongly regulated (Table 4). Concentrations of K^+ in hemolymph of shrimp from all treatments remained similar regardless of water ionic profile or feed K^+ level. Such results are not surprising given the importance of K^+ for muscular and neuronal function and maintenance of transmembrane potentials in all cells.

There were no differences in survival, growth, or FCR among treatments in trial 2 (Table 5). Arginine supplementation did not increase growth or survival of Pacific white shrimp. Potassium in the diet did not cause an increase in K^+ levels in the hemolymph of the shrimp. However, K^+ (~8 milliequivalents/L) levels were strongly regulated above ambient water K^+ levels. Similar results were observed by Dall and Smith (1981) in four species of Australian penaeid shrimp. However, Dall and Smith (1981) maintained their shrimp in low-salinity oceanic water, where the Na:K and Cl:K ratios were more appropriate for shrimp regulation. Another hemolymph property strongly regulated by shrimp in

TABLE 4:—Selected mineral content of whole Pacific white shrimp (*L. vannamei*) acid digested and measured by inductively coupled argon plasma spectrophotometry. Shrimp were reared in a high- or low-stress farm environment in Alabama and were fed diets with or without K^+ supplementation. Based on the Student–Newman–Keuls test, values in the same column with the same letter are not significantly different ($P \leq 0.05$; PSE = pooled SE).

Environment (diet) or statistic	Ca (g/100 g)	K (g/100 g)	Mg (g/100 g)	P (g/100 g)	Al (mg/kg)	Mn (mg/kg)	Na (mg/kg)
Low stress (basal)	0.73 z	0.24 z	0.030 z	0.15 z	7.9 z	2.2 z	1,932.0 z
Low stress (K)	0.60 y	0.24 z	0.030 z	0.16 z	3.83 z	1.7 z	1,777.0 y
High stress (basal)	0.61 y	0.23 z	0.037 y	0.16 z	11.3 z	4.6 y	1,556.0 x
High stress(K)	0.64 yz	0.24 z	0.035 y	0.17 z	18.4 y	6.75 x	1,626.0 x
P-value	0.03	0.16	0.0038	0.27	0.0026	<0.0001	0.0004
PSE	0.030	0.0041	0.0013	0.007	2.10	0.34	45.5

TABLE 5.—Average initial weight, final weight, survival (%), feed conversion ratio (FCR = feed offered/biomass harvested), hemolymph osmolality, and hemolymph K^+ concentration of Pacific white shrimp juveniles reared in tanks in a low-stress farm environment in Alabama for 9 weeks and offered feed supplemented with an amino acid–K complex or arginine. No differences were observed among treatments (PSE = pooled SE).

Treatment or statistic	Initial weight (g)	Final weight (g)	% survival	FCR	Osmolality (mmol/kg)	K^+ (mEq/L)[a]
0.0% K^+ in feed	0.34	15.2	76	1.4	660.0	8.13
0.5% K^+ in feed	0.34	16.5	71	1.4	647.3	7.74
1.0% K^+ in feed	0.34	16.4	70	1.6	651.5	7.56
Arginine in feed	0.34	16.0	76	1.4	651.0	—
Culture water value					52.0	0.31
P-value	0.78	0.46	0.90	0.85	0.56	0.32
PSE	0.002	0.62	7.44	0.15	6.38	8.20

[a] Milliequivalents/L.

the present experiment was osmolality. Shrimp maintained hemolymph osmolality at about 650 mmol/kg, which is much higher than the 52 mmol/kg of culture water. Similar regulation was reported by Gong et al. (2004) in Pacific white shrimp and by Parado-Estepa et al. (1987) in Indian white shrimp *Penaeus indicus*. Crustacean osmotic adaptations range from strong osmoconformity to strong osmoregulation (see Péqueux 1995), and penaeids appear to be among the strong osmoregulators, although degree of osmoregulation changes with species (see Dall and Smith 1981). Our results suggest that the Pacific white shrimp is a strong osmoregulator, but that should not be mistaken for euryhalinity. Although Pacific white shrimp are generally tolerant of a wide range of salinities, the minimum salinity tolerance depends on strain (Saoud et al. 2003), ionic composition of culture water (Zhu et al. 2004; Davis et al. 2005; Roy 2006), acclimation of postlarvae (Saoud et al. 2003; McGraw and Scarpa 2004), diet and highly unsaturated fatty acids (Palacios et al. 2004), and temperature (Tsuzuki et al. 2000). Nonetheless, adding K^+ to feeds of shrimp reared in inland LSWW does not seem to affect osmoregulation.

Although no statistically significant effects of amino acid chelated K^+ supplementation to the diet were observed, our results and those of two other experiments conducted in our laboratory under controlled conditions (Roy 2006; Roy et al. 2007) show an increase in growth associated with chelated K^+ supplementation. A 1.0% chelated K^+ supplement in the diet achieved better shrimp growth, but not better survival, when compared with NaCl, $MgCl_2$, and 0.5% chelated K^+ supplements (Roy et al. 2007). However, the use of KCl as a dietary supplement failed to yield a positive effect on growth, survival, or osmoregulation in Pacific white shrimp. This would indicate a marginal benefit for the use of a chelated K^+ supplement. A reason for this observation might be that dietary K^+ helps to reduce osmotic stress for only short periods after assimilation. Potassium uptake via the gut is subsequently lost to the ambient water, and shrimp need to expend energy in maintaining a hemolymph K^+ level that is much greater than concentrations in the water. Another explanation for the lack of results under outdoor conditions could be that some K^+ is obtained from natural foods sources, which helps offset a marginal deficiency. Irrespective of the reason for the marginal response, these results do not justify dietary K^+ supplementation for shrimp reared in inland low-salinity water. Better shrimp survival and growth are observed when K^+ is added to the culture waters to improve the Na:K or Cl:K ratio.

Acknowledgments

We are grateful to the Alabama Agricultural Experiment Foundation for providing funds for this study. Thanks also to Nelson Sansing for technical assistance with this study. The mention of trademarks or proprietary products does not constitute an endorsement of the product by Auburn University and does not imply its approval to the exclusion of other products that may also be suitable.

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