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[Technical Note]

Effects of Betaine Supplementation to Feeds of Pacific White Shrimp *Litopenaeus vannamei* Reared at Extreme Salinities

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Abstract.—Shrimp production worldwide has increased dramatically, and optimal sites are no longer abundant. New farms are being constructed in areas where water salinity and ion composition are suboptimal. Aquaculturists and feed suppliers are attempting to alleviate ion nonequilibriums through nutrition. One nutritive supplement that has been marketed is the amino acid betaine. The present work evaluated the effects of betaine as a feed supplement on the survival and growth of Pacific white shrimp *Litopenaeus vannamei* reared at extreme salinities (0.5 or 50‰). Juvenile Pacific white shrimp (mean individual weight, 0.18 g) were reared in 16 tanks: eight tanks held water at 0.5‰, and eight held water at 50‰. Shrimp were maintained for 8 weeks in four replicate tanks from each salinity treatment and offered feed with or without a betaine supplement. Survival (75–89%) and final weights (2.8–3.5 g) were typical for this species reared in indoor systems, but there was no significant influence of the presence of betaine. However, there was a significant influence of salinity on growth. These results suggest that betaine supplementation to practical diets designed for Pacific white shrimp does not improve production at extremely low or high salinities.

World shrimp production from aquaculture has increased to over 4 million metric tons, accounting for more than 25% of total world shrimp production (Josupeit and Jacobsen 2003). The continued expansion of the industry has favored the development of new aquaculture projects in areas where water quality is not always appropriate for the optimal development of fish or shrimp. This is particularly true for the production of marine shrimp, when farms are located inland using groundwater of varying salinities or when farms are located near estuaries that may have very high or very low water salinities. Penaeid species of shrimp have differing tolerances to high or low salinities (Zein-Eldin 1963; Valencia 1976; Mair 1980; Parado-Estepa et al. 1987; Harpaz and Karplus 1991; Rosas et al. 1999; Tsuzuki et al. 2000; Saoud and Davis 2003). These tolerances can be used when determining the species to be used at a specific site. Although shrimp may tolerate these environments, growth is potentially compromised. The ions most important in osmoregulation are chloride and sodium (Castille and Lawrence 1981; Ferraris et al. 1986; Parado-Estepa et al. 1987). However, at very high or very low salinities, osmolality cannot be maintained within zones of tolerance by Cl^- and Na^+ alone. Free amino acids (FAAs) in the cells participate in maintaining intracellular osmolalities within acceptable levels (Schoffeniels 1970; Gerard and Gilles 1972). Various FAAs (such as proline, glycine, arginine, and taurine, among others) have been shown to affect osmoregulation in various tissues of aquatic organisms (Schoffeniels 1970; Gerard and Gilles 1972; Marangos et al. 1989; Deaton 2001). Another FAA that is important in intracellular osmoregulation is betaine. Betaine has been identified as a chemoattractant that elicits a feeding response in crustacea (Carr et al. 1984) and assists in the osmoregulation of aquatic organisms at extremely high salinities (Virtanen et al. 1994; Deaton 2001; de Vooys and Geenevasen 2002). Hence, betaine supplementation to feeds of shrimp maintained at extreme salinities may improve survival and growth by helping shrimp maintain osmotic balance. In the present experiment, we evaluated the effects of betaine supplementation to a practical shrimp feed on the survival and growth of Pacific white shrimp *Litopenaeus vannamei* maintained in extreme salinities.

Methods

The present research was performed at the North Auburn Fisheries Research Station, Auburn University, Auburn, Alabama. Two batteries of eight square polyethylene tanks ($60 \times 60 \times 45$ cm) were connected to a biological filter and a reservoir tank. Both batteries were filled with reconstituted seawater at 10 ppt and each tank stocked with 20 juvenile Pacific white shrimp (0.18-g mean individual weight) acclimated to 10‰. The salinity in one battery was then gradually reduced to 0.5‰ over 1 week by adding fresh groundwater. The salinity in the second battery was raised to 50‰ over 1 week by adding a brine solution of recon-

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TABLE 1.—Ingredient composition of experimental diets (g/100 g dry weight) fed to Pacific white shrimp for 6 weeks under controlled conditions.

Ingredient	Diet 1	Diet 2
Menhaden fish meal[a]	6.0	6.0
Poultry meal[b]	15.0	15.0
Soybean meal[c]	32.0	32.0
Menhaden fish oil[d]	3.6	3.6
Wheat starch[e]	2.31	2.28
Whole wheat[e]	36.0	36.0
Trace mineral premix[f]	0.5	0.5
Vitamin premix[g]	2.0	2.0
Vitamin C[h]	0.07	0.07
Calcium phosphate dibasic[e]	1.4	1.4
Betaine[i]	0.0	0.4
Caseine[e]	0.92	0.55
Cholesterol[e]	0.2	0.2
Total protein	36%	36%
Total lipid	7.1%	7.1%

[a] Omega Protein, Inc., Hammond, Louisiana.
[b] Griffin Industries, Inc., Cold Springs, Kentucky.
[c] Dehulled solvent-extracted soybean meal; Southern States Cooperative, Inc., Richmond, Virginia.
[d] Omega Protein, Inc., Reedville, Virginia.
[e] United States Biochemical Corporation, Cleveland, Ohio.
[f] Composition (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] Composition (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 B_{12}, 0.002; choline chloride, 100.0; inositol, 5.0; menadione, 2.0; vitamin A acetate (20,000 IU/g), 5.0; vitamin D_3 (400,000 IU/g), 0.002; DL-alpha-tocopheryl 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), Roche Vitamins, Inc., Parsippany, New Jersey.
[i] ICN Biochemicals, Inc., Aurora, Ohio.

stituted seawater. Shrimp in four of the low salinity tanks and four of the high salinity tanks were offered a practical diet with no betaine supplement, while shrimp in the remaining tanks were offered the same feed with a 0.4% supplement of betaine (Table 1). Shrimp were counted weekly and ration was calculated assuming a 1.75 feed conversion ratio (weight of food fed/weight gained) and a doubling in size until individual shrimp weighed 1 g, and a growth rate of 1 g/week thereafter. At the end of an 8-week growth period, shrimp were harvested, counted, and group weighed.

The experiment was set up as a 2 × 2 factorial design, salinity and betaine supplementation being the variables. Statistical analyses were performed using SAS (SAS Institute 2002). Effects of salinity and dietary betaine supplementation on survival and growth were evaluated by two-way analysis of variance (ANOVA; $P \leq 0.05$).

TABLE 2.—Initial weight, final weight, and percent survival of Pacific white shrimp maintained at two salinities and offered feeds with and without betaine supplements.

Salinity (‰)	Betaine in diet	Initial weight (g)	Final weight (g)	Survival (%)
0.5	Yes	0.18	3.5	81
0.5	No	0.18	3.4	75
50	Yes	0.18	2.8	88
50	No	0.18	2.9	89

Results and Discussion

The rapid growth of shrimp aquaculture is causing farmers to use farm sites with waters traditionally considered unsuitable because of extreme water salinities. Some feed manufacturers suggest that betaine supplementation to feeds will help osmoregulation of shrimp reared under unsuitable salinity conditions. However, betaine inclusion in shrimp feeds in the present experiment did not affect survival and growth at either a very low (0.5‰) or a very high (50‰) salinity (Tables 2, 3). Although betaine has been shown to improve osmoregulation in various aquatic organisms (see de Vooys and Geenevasen 2002), no effect of interaction between salinity and betaine inclusion was detected in this study. Accordingly, our results do not support claims of improved osmoregulation and survival in Pacific white shrimp fed diets containing a betaine supplement. Other FAAs—such as proline, glycine, arginine, and taurine—also affect osmoregulation in various aquatic organisms (Schoffeniels 1970; Gerard and Gilles 1972; Marangos et al. 1989; Deaton 2001) and in various tissues within the same organism. Osmoregulation in Pacific white shrimp might be assisted by FAAs other than betaine.

Conversely, in the present study salinity did have an effect on survival and growth. Shrimp reared at 50‰ survived better but grew slower than shrimp reared at 0.5‰ (Tables 2, 3). The fact that shrimp in the present experiment performed better at 0.5‰ than at 50‰ does not imply that Pacific white

TABLE 3.—Effects of salinity, betaine supplementation, and interaction of the two variables as analyzed by two-way ANOVA.

Factor	*F*-statistic		
	Initial weight (g)	Final weight (g)	Survival (%)
Salinity	0.512	0.001	0.020
Treatment[a]	0.825	0.805	0.514
Salinity × treatment	0.825	0.505	0.333

[a] Treatment = inclusion of betaine in diet.

shrimp grow better at low salinities. It should be noted that there are several strains of Pacific white shrimp and they tend to have different salinity tolerances (Harpaz and Karplus 1991; Kumulu and Jones 1995). Bray et al. (1994) suggest that the Ecuadorian strain of Pacific white shrimp (used in the present work) grows better at low salinity than the Mexican strain. Farmers should ask hatchery managers about the strain of shrimp they produce and the salinity preferences of those strains before purchasing postlarvae to stock ponds.

The present experiment demonstrated that betaine supplementation to feeds of Pacific white shrimp does not improve survival or growth when the shrimp are reared at extremely high or low salinities. However, such results cannot be extrapolated to other penaeid species. Various species use different intracellular osmoregulatory compounds which should be identified on a species-by-species level. Thereafter, feeds that assist in maintaining osmotic balance within zones of tolerance for each species can be developed.

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