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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.

Keywords: Low salinity; Pacific white shrimp; Cholesterol; Phospholipid

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 ($CaCl_2 \cdot 2H_20$). 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 ± 0.34 mg L^{-1}), temperature (28.6 ± 1.0 °C), pH (8.1 ± 0.1), salinity (4.1 ± 0.04 g L^{-1}), ammonia (0.03 ± 0.02 mg L^{-1}), and nitrites (0.14 ± 0.18 mg L^{-1}) remained within acceptable limits