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Intensive nursery production of the Pacific white shrimp *Litopenaeus vannamei* using two commercial feeds with high and low protein content in a biofloc-dominated system

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ABSTRACT

The efficiency of shrimp production in limited exchange systems can be improved by optimizing the protein content of the feed. Therefore, a 62-d nursery study was conducted with 10-day-old *Litopenaeus vannamei* postlarvae stocked (5000 PL_{10} m^{-3}) in four 40 m^3 raceways to evaluate the effect of high (40%) and low (30%) crude-protein (HP40 and LP30, respectively) diets and molasses supplementation on selected water quality indicators and shrimp performance under limited water exchange. Each raceway was equipped with a real-time dissolved oxygen monitoring system and a foam fractionator to control particulate matter. The level of molasses used in this study was effective in preventing significant ammonia accumulation in the culture medium. However, these supplementation levels were not effective in preventing nitrite accumulation. The HP40 treatment had significantly higher nitrite, nitrate and phosphate concentrations than the LP30 treatment. Shrimp mean final weight (0.94 vs. 1.03 g) and specific growth rate (SGR) (11.03 vs. 11.19% day^{-1}) were significantly different between treatments ($P < 0.05$) LP30 and HP40, respectively, while mean survival (~82% vs. 84%) and protein efficiency ratio (PER) (3.89 vs. 3.28) were not ($P > 0.05$). The data suggest that substituting high-protein (40%) with low-protein (30%) feed in the nursery phase in a biofloc dominated system operated with minimal discharge may provide an alternative to improve shrimp biofloc technology, through improved water quality, cheaper (lower protein) feed and reduced environmental impact.

1. Introduction

Environmental damage associated with effluent discharge and massive crop losses due to disease outbreaks have created a need for more sustainable and biosecure shrimp production practices (Cowey and Cho, 1991; Samocha, 2009). Implementation of limited or no water exchange shrimp production systems has the potential to minimize these negative environmental impacts and disease outbreaks, while conserving water resources and not compromising profit. Several studies have shown that the Pacific white shrimp, *Litopenaeus vannamei*, can be cultured with reduced water exchange with no adverse affect on growth, survival and yield in the nursery and grow-out phases (Samocha et al., 1998; Moss et al., 1998; Cohen et al., 2005; Mishra et al., 2008; Samocha, 2009).

The output of intensive limited discharge systems can be improved when a nursery phase is included in the production scheme (Samocha, 2009, 2010). The shrimp nursery phase is defined as an intermediate step between the young postlarval (PL) stage and the grow-out phase. As nursery systems are stocked at high densities, this practice can improve facility utilization and provide better control over water quality and feed management that can lead to improved shrimp performance and profit in the grow-out phase (Sturmer et al., 1992; Samocha and Lawrence, 1992; Yta et al., 2004). Using greenhouse-enclosed limited-exchange systems can also be beneficial for shrimp nursery production in temperate climate areas to accommodate PL early season stocking ("headstart"), when the ambient water temperature in grow-out ponds is too low for the shrimp to survive and/or

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grow. This practice can extend the grow-out season to produce larger shrimp or to grow multiple crops per year (Samocha et al., 2000a,b; Samocha and Benner, 2001; McAbee et al., 2003).

There is a need to develop diets for shrimp cultured in limited exchange nursery systems that will provide sufficient protein for shrimp production while minimizing the amount of nitrogen being introduced into the culture medium (McIntosh et al., 2001). Shrimp typically have a higher dietary protein requirement during the nursery phase than at later stages (Chen et al., 1985; Velasco et al., 2000). However, there is a wide range in reported dietary protein requirements for *L. vannamei*, typically from 300 to 480 g kg^{-1} (30–48%), with an optimum for PL of 340 g kg^{-1} (34%) (Hu et al., 2008). In intensive nursery systems *L. vannamei* have been fed diets with protein levels as high as 40–55% (Samocha et al., 1993; Velasco et al., 2000).

The effluent water from intensive shrimp production systems often has high loads of nitrogen (N), phosphorus (P), particulate organic and inorganic matter, and oxygen demand (Cohen et al., 2005). Much of the nitrogen input in culture systems enters the water column as total ammonia-nitrogen generated by feed which is not converted into shrimp tissue. Thakur and Lin (2003) showed that under no exchange *Penaeus monodon* assimilated only 23–31% of the nitrogen added to the system. The presence of microbial and algal communities in limited discharge systems helps with the recycling of the system's metabolites (Avnimelech, 1999; Burford et al., 2003; Wang, 2003). Besides the nutrient recycling aspect, the dense bacterial community that develops in such systems plays a significant role in the production of single cell microbial protein ("biofloc") that can provide supplemental natural feed for the shrimp (Avnimelech et al., 1994; Avnimelech, 1999; Browdy et al., 2001). Wasielesky et al. (2006) suggested that this enhanced natural production in zero exchange production systems allows the use of low protein feeds with no adverse effect on shrimp performance compared to high protein feeds.

As feed is the major driving force of intensive production systems, it is important to optimize its use to improve profitability, maximize growth, and minimize potential water quality deterioration. With this in mind, a 62-d trial was conducted with Pacific white shrimp, *L. vannamei*, PL under limited water exchange to improve feed management and water quality and optimize protein efficiency during the nursery phase. Specifically, the study had two major objectives: (1) to determine the effect of substituting high-protein (40%) with low-protein (30%) feed on shrimp growth, survival, protein efficiency ratio and selected water quality indicators in a biofloc-dominated system, and (2) to determine if molasses can be used to prevent ammonia and nitrite accumulation in a super-intensive shrimp nursery system.

2. Materials and methods

2.1. Site and experimental system

The study was carried out in four 40 m^3 ethylene propylene diene monomer (EPDM, Firestone Specialty Products Company, Indianapolis, Indiana) lined greenhouse-enclosed raceways (RWs) at the Texas A&M AgriLife Research Mariculture Laboratory at Flour Bluff, Corpus Christi, Texas. Each RW (68.5 m^2 bottom area; 25.4 m × 2.7 m) had a center longitudinal fiberglass partition positioned over a 5.1 cm PVC pipe with spray nozzles every 0.5 m. Every RW had six banks, each with three 5.1 cm airlift pumps, and six 0.92 m long air diffusers (1.9 cm OD, Aero-Tube™, Tekni-plex Aeration, Austin, Texas). Airlifts and air diffusers were positioned at equidistant on both sides of the partition and were operated continuously using a 3 HP regenerative air blower (Rotron, DR404, Area Inc., Homestead, FL) and a 7.5 HP positive displacement air blower (Model 4007-21L2, Tuthill Vacuum & Blower System, Houston, TX). In addition, each RW had a 2 HP centrifugal pump (Hydrostorm, Waterco Inc., Augusta, GA) and a Venturi injector (Model MIC-1583A, Mazzei Injector Co., Bakersfield, CA). To increase dissolved oxygen levels in the culture medium, the Venturi injector was set to operate using atmospheric air, pure oxygen or a mixture of the two. Water circulation through the nozzles on the pipe under the center partition was initiated on Day 17 with 20 min of operation in the morning and 40 min in the afternoon. This water circulation was increased gradually so that by Day 35 it was operated continuously. Each RW was equipped with an inline dissolved oxygen monitoring and alarm system (YSI 5200 multi-parameter system, Yellow Springs Instruments, Yellow Springs, OH). To control the levels of particulate matter, every RW had a small commercial foam fractionator (Model VL65, Aquatic Eco-systems, Inc., Apopka, FL) which was run periodically.

Prior to filling, RWs were sprayed with 500 ppm chlorinated freshwater. The trial was done with natural seawater from Upper Laguna Madre adjusted to 30 ppt salinity using municipal freshwater. Culture water was chlorinated to reach 10 ppm of active chlorine concentration 30 min after chlorination. Chlorine was removed by aeration only. Two days before stocking, water was fertilized using urea, phosphoric acid and sodium silicate to provide concentrations of 2.62 mg L^{-1}, 0.25 mg L^{-1} and 1.66 mg L^{-1} for N, P, and Si, respectively. On the day of stocking RWs water was inoculated with *Chaetoceros muelleri* to provide an initial algal concentration of 70,000 cells mL^{-1}.

2.2. Stocking and culture management

All four RWs were stocked with 10-day-old postlarvae (PL_{10}, ~1 mg) at a density of 5000 m^{-3}. Postlarvae were produced from a specific-pathogen-free breeding population by Harlingen Shrimp Farms, Ltd., Los Frenos, TX. Each RW received 500 mL of molasses (24% carbon, specific gravity of 1.3, according to Samocha et al., 2007) every other day from Day 10 through Day 18 to promote development of heterotrophic bacteria. From Day 19 on, molasses was added to give 6 g of organic carbon for every 1 g of total ammonia nitrogen (TAN) found in the culture medium (Samocha et al., 2007). No molasses was added from Day 30 until the end of the trial as TAN concentrations were consistently below 0.5 mg L^{-1}. Salinity was maintained at about 30 ppt by adding freshwater to offset losses due to evaporation. Some new water was also added to offset water loss associated with the use of the foam fractionator. Shrimp were sampled twice a week to monitor health, growth, and to adjust daily rations.

2.3. Feeds and feeding

For the first four days after stocking PL in all RWs were fed newly hatched live *Artemia* nauplii (~40 PL^{-1} day^{-1}), and a combination of dry diets consisting of PL Redi-Reserve (400–600 μm, Zeigler Bros., Inc., Gardners, PA), SureStart #3 (300–500 μm), SureStart #4 (500–800 μm) (55% CP, Salt Creek, Inc., Salt Lake City, UT), 40% CP Fry #0 (420–590 μm), and Fry #1 (600–1000 μm) (Rangen Inc., Buhl, ID). This mixture of dry feeds was offered until Day 26. Beginning on Day 27, once biofloc was established, shrimp were fed two commercial diets made by Rangen Inc., with one containing 30% CP (LP30) fed to two RWs and one containing 40% CP (HP40) fed to the other two RWs (Table 1). These two diets were fed to shrimp as Fry #2 (1.0–1.41 mm) from Day 27 through 46, followed by Fry #3 (1.41–1.68 mm) from Day 47 through 59, and Fry #4 (1.68–2.83 mm) from Day 60 through 61. The transitions from one particle size to the next were done gradually, based on shrimp ability to handle the different particle sizes. Feeding rates ranged from 50% of the total estimated biomass (0.5 mg feed $shrimp^{-1}$ day^{-1})