Aquacultural Engineering 67 (2015) 53–58

Contents lists available at ScienceDirect

Aquacultural Engineering

journal homepage: www.elsevier.com/locate/aqua-online

Intensive hatchery performance of the Pacific white shrimp in biofloc system

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ARTICLE INFO

Article history:
Received 14 February 2015
Received in revised form 8 May 2015
Accepted 29 May 2015
Available online 3 June 2015

Keywords:
Zero-exchange
Larvae
Heterotrophic
BFT
Intensive culture

ABSTRACT

We assessed the hatchery performance of *Litopenaeus vannamei* between the mysis1 and postlarva 5 stages, in a zero-exchange biofloc system. Two sources of organic carbon (molasses and dextrose) were evaluated and water quality, zootechnical parameters, microbiology, and water consumption during production were compared between carbon-supplemented and control groups. The mean values of the evaluated water quality parameters were appropriate for this production stage. Fertilization with molasses and dextrose efficiently controlled ammonia levels and ammonia did not reach the average concentrations that are considered toxic for the species (total ammonia < 1.3 mg L^{-1} and free ammonia < 0.05 mg L^{-1}). The number of heterotrophic bacteria in the water was greater in the molasses and dextrose groups than in the control group. However, there was no difference in Vibrionaceae count between groups. There was no difference between groups in survival (>85%), length (6.15 mm), dry weight (0.17 mg) of postlarvae 5. Treatment with dextrose or molasses required approximately 12% of the water used by the control group. *L. vannamei* production rates and water quality were maintained without water exchange using a biofloc system supplemented with dextrose or molasses.

1. Introduction

The hatchery stage is a critical stage in the production of Pacific white shrimp (*Litopenaeus vannamei*), during which strict quality criteria and constant attention are required. The hatchery stage extends from the nauplii phase to the postlarva 5 (PL5) phase. At this stage, shrimp are extremely susceptible to physical, chemical, and biological stressors, such as vibriosis outbreaks (Vandenberghe et al., 1999; Aguirre-Guzman et al., 2001; Mourino et al., 2008; Martin et al., 2012). In order to minimize losses due to disease and environmental impacts, hatchery systems with biofloc technology (BFT) and reduced water exchange have been developed (Samocha et al., 2007). Intensive shrimp production is traditionally performed in a predominantly autotrophic medium, with high rates of daily water exchange (Wang, 1990). At this stage, microalgae rich in polyunsaturated fatty acids are added every day, in addition to the water renovations (Piña et al., 2006; Ju et al., 2009; Khatoon et al., 2013). These microalgae exchanges not only contribute to the nutrition, but also enable the control of ammonia nitrogen levels in the farming tanks (Ebeling et al., 2006). Negative impacts associated with such production systems, such as the discharge of large volumes of water containing high levels of ammonia nitrogen and phosphorus (microalgae, feces, and uneaten feed) may threaten coastal ecosystems and lead to health risks due to self-pollution (Hopkins et al., 1995; Samocha et al., 2007). In this context, the sometimes-considerable economic costs of the energy required to capture, heat, and distribute large volumes of water must be considered.

In an attempt to minimize the impact of the environmental, health, and economic problems associated with shrimp aquaculture, BFT has become increasingly common (Avnimelech, 1999, 2006; Browdy et al., 2001; Crab et al., 2007; De Schryver et al., 2008). BFT is used to intensify production and avoid the exchange of farming water, with a consequent reduction in the flow of pathogens and

Abbreviation: BFT, biofloc technology.

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http://dx.doi.org/10.1016/j.aquaeng.2015.05.007
0144-8609/

discharge of nutrient-rich effluents into the environment (Samocha et al., 2007).

Reducing or ceasing water exchange requires control of the ammonia that results from protein catabolism, as it is toxic to fish and shrimp. Ionized and non-ionized ammonia are present in the water of aquaculture tanks in variable proportions that are influenced by factors such as pH, temperature, and salinity. The non-ionized form of ammonia is more toxic to shrimp than the ionized form, and causes a variety of physiological damage, due to its affinity for the non-polar compounds of the plasma membrane (Bower and Bidwell, 1978; Chen et al., 1996; Lin and Chen, 2001).

In BFT systems without water exchange, the ammonia control strategy centers on the establishment of a carbon–nitrogen balance that facilitates the growth of heterotrophic bacteria, which incorporate ammonia nitrogen from the medium (Moriarty, 1997; Avnimelech, 1999; Ebeling et al., 2006; Hari et al., 2006; Samocha et al., 2007). This relationship is established by adding organic carbon sources (molasses, flours, sugar, and dextrose) to aquaculture media. It requires 20 g of carbohydrate, or about 6 g of carbon, to convert 1 g of ammonia nitrogen to bacterial biomass (Avnimelech, 1999; Ebeling et al., 2006). In BFT culture systems, chemoautotrophic and heterotrophic bacteria participate in the formation of bioflocs, which also include an aggregate of algae, fungi, protozoa, rotifers, and nematodes (De Schryver et al., 2008). Therefore, in addition to providing ammonia control, bioflocs may represent a food source in farming tanks (Avnimelech, 1999; Cuzon et al., 2004).

The use of BFT systems in the pre-nursery and fattening stages of marine shrimp aquaculture, have been extensively studied (Emerenciano et al., 2011, 2012, 2013; Ray et al., 2011; Xu and Pan, 2012; da Silva et al., 2013; Schveitzer et al., 2013; de Souza et al., 2014; Kumar et al., 2014). However, no systematic studies have been published showing BFT without water exchange during the hatchery phase as a viable alternative to the standard production system of penaeid shrimp larvae. The hatchery stage starts at mysis1 (M1) and continues through the PL5 phase. Daily water exchanges are typically performed throughout this stage. The objective of this study was to assess the hatchery performance of *L. vannamei* that were reared between M1 and PL5 using a biofloc system supplemented with organic carbon (molasses or dextrose) and without water exchange.

2. Materials and methods

The experiment was conducted at the Laboratório de Camarões Marinhos (LCM), Departamento de Aquicultura da Universidade Federal de Santa Catarina, Brazil.

2.1. Biologic material

Before the experiment, nauplii of *L. vannamei* were raised in a 20 m^3 (stocking density of 100 larvae L^{-1}), semi-cylindrical hatchery tank in salinity of 35 ppm until they reached mysis1. The microalgae *Chaetoceros muelleri* (5×10^4 cells mL^{-1}) was added to the culture water daily. When the larvae reached the stage of M1 (average dry weight of 0.085 ± 0.004 mg and average length of 3.543 ± 0.076 mm) they were transferred to the experimental units, which were initially filled with water from the hatchery tank. This line was free of any pathogens that require notification of the International Organization of Epizootics (from Aquatec LTDA, Rio Grande do Norte, Canguaratema, Brazil).

2.2. Experimental conditions

Three groups of larvae were prepared: two experimental groups were reared in a heterotrophic system without water exchange and a control group was reared in a conventional autotrophic system with daily water exchange and the addition of microalgae. The organic source was added to the culture water of the two experimental groups. Anhydrous dextrose ($C_6H_{12}O_6$, Sigma–Aldrich®) was added to the culture water of one group and sugar cane molasses (55% carbohydrate, 3% crude protein) was added to the culture water of the second group.

The experimental groups were randomly distributed in a unifactorial experimental design. Semi-cylindrical plastic tanks (92 cm × 68 cm × 25 cm) with a working volume of 60 L constituted the experimental units. Four tanks were prepared for each experimental condition, resulting in 12 tanks. All tanks were equipped with linear aeration supplied by a PVC pipe (90 cm long, 20 mm diameter with 36 holes of 1 mm) to keep the solids generated during cultivation in suspension and maintain the level of dissolved oxygen in the water at the recommended concentration for *L. vannamei* larval cultivation (>5 mg L^{-1}). The water temperature was kept constant, between 29 and 30 °C, using 100-W heaters connected to a thermostat.

The experimental tanks were supplied with water from an autotrophic larval cultivation. The water supplied had the following parameters: *C. muelleri* (5×10^4 cells mL^{-1}), oxygen = 5.19 mg L^{-1}, pH = 7.92, temperature = 31.65 °C, salinity = 35.57 mg L^{-1}, total ammonia = 0.92 mg L^{-1}, free ammonia = 0.05 mg L^{-1}, nitrite = 0.01 mg L^{-1}, nitrate = 1.56 mg L^{-1}, phosphate = 0.187 mg L^{-1}, total suspended solid (TSS) = 170.1 mg L^{-1}, volatile suspended solid (VSS) = 42.7 mg L^{-1}, and alkalinity = 132 mg L^{-1}.

Each experimental unit was stocked with 12,000 larvae in M1, which represents a stocking density of 200 larvae L^{-1}. The experiment was conducted until the larvae reach the postlarvae stage 5 (7 days after stoking). The water in the biofloc experimental units was not exchanged during the experimental period, but evaporated water was replaced with fresh water in order to maintain salinity. No suspended solids were removed from the water during the experiment. To keep total ammonia nitrogen (TAN) below the established maximum of 1 mg L^{-1}, the water of the control units was exchanged at rates that ranged from 50% per day at the start of the experiment to 200% in the final stage of cultivation. Water samples were taken from the control units after each water exchange. *C. muelleri* was counted in the water samples. In order to provide food for larvae and to maintain water quality, *C. muelleri* was added as needed to maintain a concentration of 5×10^4 cells mL^{-1}.

The larval and postlarval shrimp were fed microencapsulated commercial diets (INVE). The Lansy ZM diet (minimum protein 48%, minimum ether extract 13%, maximum fibrous matter 2.5%, maximum mineral matter 13%, maximum humidity 8.0%, minimum calcium 0.25%, maximum calcium 1.5%, and minimum phosphorus 1.0%) was fed from M1 to mysis3/postlarva 1. After this period, and until harvesting, postlarvae were fed the Lansy MPL diet (minimum protein 48%, minimum ether extract 9.0%, maximum fibrous matter 2.5%, maximum mineral matter 13%, maximum humidity 9.0%, minimum calcium 1.0%, maximum calcium 2.2%, and minimum phosphorus 1.0%). Larvae were fed nine times a day (0800, 1000, 1200, 1400, 1600, 1800, 2100, 2300, and 0300) and were provided INVE quantities according to the manufacturer's recommendation for each larval stage. *Artemia* nauplii were also provided to the larvae at a rate of six nauplii for each mysis or postlarva, five times each day (0900, 1100, 1500, 1700, and 0000).

2.3. Addition of carbohydrates

Sources of organic carbon were added (divided in four times per day) to the biofloc treatment tanks (dextrose or sugar cane molasses) to maintain ammonia levels of <1 mg L^{-1}. The percentage of carbohydrate was assumed to be 100% for dextrose and 55% for molasses.