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Effect of commercial probiotics addition in a biofloc shrimp farm during the nursery phase in zero water exchange

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ABSTRACT

In biofloc technology systems (BFT) the bacterial community plays the most important role at recycling the organic matter and metabolizing the toxic nitrogenous compounds. The aim of this study was to evaluate the effect of two groups of commercial probiotics on the abundance of viable heterotrophic bacteria (VHB), ammonia oxidizing bacteria (AOB) and *Vibrio*-like (VLB), nitrogenous compounds and productive parameters of whiteleg shrimp *Litopenaeus vannamei* in a commercial farm. The study was developed during nursery phase in zero water exchange. Two groups of probiotics (PB1 and PB2) and one control (PBN; no probiotic) were evaluated in three replics. Shrimp postlarvae (7.3 mg) were stocked in ponds (70 m^3) at densities of 500 in./m^2. Commercial probiotics (Efinol PT-Lab. Robles and Epicin ponds-Epicin hatcheries) were incubated for 36 h in bioreactors (1 m^3) and supplied every 3 d in PB1 and PB2 treatments. Basic variables of water quality, nitrogenous compounds and bacterial groups were monitored. At the beginning of the experiment, the abundance of VHB was lower in the PBN treatment, but as the bioassay progressed, the densities were similar to PB1 and PB2. In general VLB showed similar tendency to VHB, but significant difference were determined among treatments. The AOB abundance was similar in all treatments. Nitrogenous compounds and productive parameters of shrimp did not show significant differences between treatments. In the PBN ponds the zero water exchange could have promoted benefical bacteria of the natural biota. The addition of probiotics did not improve the water quality nor productive response of *L. vannamei*.

1. Introduction

The whiteleg shrimp (*Litopenaeus vannamei*) is the most cultured species along the coastal zones; therefore it has great social, economic and environmental importance. In recent years, the viral and bacterial diseases as well as environmental problems of water quality, have affected the shrimp farms causing pronounced economic losses (Sánchez-Paz et al., 2014; Hernández-Llamas et al., 2016). In order to reduce the shrimp diseases, the commercial farms started to culture shrimp in a hyper-intensive systems, named as biofloc technology systems (BFT). The hyper-intensive systems are considered an environmentally friendly alternative to culture aquatics species in high density with limited water exchange (Vinatea et al., 2010; Krummenauer et al., 2011).

Under adequate management (carbon: nitrogen ratio), the zero water exchange conditions, cause an organic matter and nutrients accumulation into the culture system. It naturally promotes the development of microbial community. The high diversity of microorganisms plays an important role in stabilizing the system, included: maintenance of water quality, by the uptake of nitrogen compounds generating *in situ* microbial protein (Burford et al., 2004; López-Elías et al., 2015); improving the nutrition (Tacon et al., 2002; Martínez-Córdova et al., 2017; Moreno-Arias et al., 2018), increasing culture feasibility by reducing feed conversion ratio and a decrease of feed costs; as well as the promotion the health of cultured organisms (Avnimelech, 2014; Aguilera-Rivera et al., 2014; Emerenciano et al., 2017).

In recent years, the hyper-intensive shrimp farms have started to use commercial microbial consortia, nevertheless, reported studies in commercial farms are scarce (Kesarcodi-Watson et al., 2008). Probiotics are defined as dietary supplements, that contain potentially benefical bacteria and which confers health benefits on the organisms FAO (2001). In aquaculture, the cultured organisms have a direct interaction

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with the environment. The natural and external microbiota are in permanent contact with the animals; considering it, Verschuere et al. (2000) suggested a new term of probiotic for aquaculture, which includes their effect over the microbial community of the water, and over the quality of the farming environment.

Several studies indicate that the probiotics could, contribute to enzymatic digestion, take part inhibiting pathogenic microorganisms, promote growth factors as well as increase immune response (Verschuere et al., 2000; Krummenauer et al., 2014). In controlled conditions (laboratory), the results seem to be highly promissory, but in commercial facilities, the environmental conditions (temperature, salinity, pH, illumination, dissolved oxygen, etc.) normally change during the day; the dynamic environment and the presence of natural bacteria, could affect effectiveness the probiotic added.

In commercial farms it is difficult to develop controlled bioassays, and frequently, the results are not conclusive; nevertheless some companies commonly use probiotics, but their effectiveness, compared with the natural bacterial populations (which can be developed naturally) is scarcely evaluated. This study contributes to generate information which can be useful to biofloc commercial shrimp farms. We sought to evaluate the hypotheses: in a BFT system, the commercial probiotic addition promotes the beneficial bacteria abundance, therefore, it can improve the water quality as well as the shrimp productive parameters. The objective was to evaluate the effect two groups of commercial probiotics used in a commercial farm over heterotrophic, Vibrio bacteria and nitrifying, as well as on settleable solids, nitrogenous compounds and productive parameters of *Litopenaeus vannamei* during the zero water exchange phase.

2. Materials and methods

2.1. Experimental design

The experiment was developed in the hyper-intensive facilities of the company Proveedora de larvas S.A. de C.V., located in Sinaloa, Mexico. The company produces whiteleg shrimp (10–12 g/ind; culture period of 90 d) in biofloc system. Their protocol consisted in two phases, the first one (nursery) is in zero water exchange (30 d) and the second one (60 d) in low water exchange (3–5% /day). The addition of probiotics is done during the entire culture cycle. To avoid interference on bacterial community by the effect of the natural microbiota, present on influent water, the study was developed during the zero water exchange (nursery phase).

This experiment was developed in 70 m^3 ponds, it consisted in a simple random design with three treatments with three replicates (commercial probiotics: PB1 and PB2, and one control PBN). The PB1 was composed by the mixture (50:50%) of Efinol PT (containing *Bacillus* spp., lactic acid, *Lactobacillus* spp., *Saccharomyces* spp.) and Mix Laboratory Robles (containing a native microbial consortia); the second, coded as PB2 was composed by the mixture (50%50%) of Epicin Ponds (the content description, indicates the presence of non-toxic natural microbial cultures, and enzymes with added stabilizers and growth stimulants) and Epicin Hatcheries (the content description indicates, the presence of non-toxic natural microbial cultures, and enzymes with added stabilizers). The control treatment coded as PBN (natural endemic microbial consortium), consisted of the traditional biofloc system without probiotics application.

2.2. Probiotic use

Two bioreactors of 1000 L (useful volume) were used to incubate the bacteria consortia. Each bioreactor received vigorous aeration by microporous tube placed in the bottom. The water (33–35 ‰) was filtered (5 microns) and sterilized with sodium hypochlorite, after 12 h was neutralized with sodium thiosulfate. The respective bioreactor were inoculated in a dose of 100 g/m^3, as carbon source 1 kg molasses/

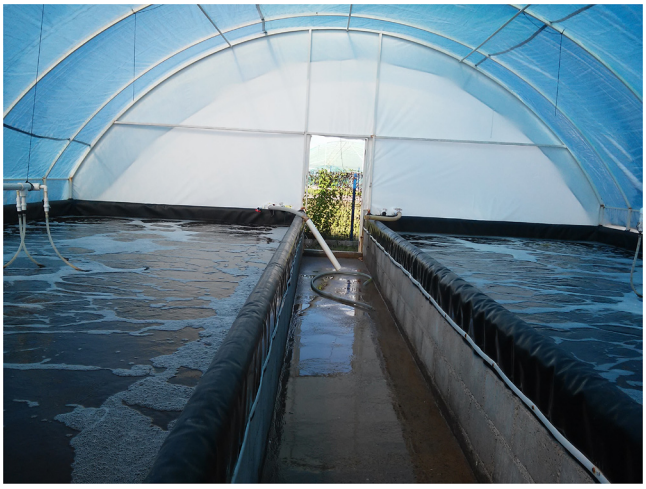

Fig. 1. Commercial facilities to culture *L. vannamei* in biofloc.

m^3 was added, as well as a mix of micronutrients with other compounds (not revealed by the company). The probiotics were incubated at 30 ± 3 °C by 36 h. Under this conditions the viable heterotrophic bacteria reached 80–120 × 10^6 CFU/mL (determined in marine agar; plate spread at 30 °C in 24 h).

The ponds of PB1 and PB2 treatments were filled 6 d previous to the shrimp stocking, while the PBN were filled 1 d previous to the shrimp stocking. The PB1 and PB2 received 70 L of probiotics each 3 days, since the filling day. The PBN treatment did not received inoculation.

2.3. Culture conditions

The whiteleg shrimp postlarvae (PL 12) were obtained from the laboratory of the same company (Proveedora de larvas S.A. de C.V.). The experimental organisms (7.3 mg) were stocked in rectangular ponds (≈70 m^3 volume; 20 m × 3 m × 1.17 m) at density of 500 in./m^2. The bottom of the ponds were covered by high-density polyethylene (HDPE). The nine experimental units were placed in the greenhouses (Fig. 1) natural photoperiod was maintained. The aeration was supplied by electric aerators in ratio of 100 Hp/Ha. Aeration grills (elaborated with porous tube) were placed in the bottom of the tanks to supply oxygen and maintain the solids in suspension.

Six days previous to the stocking, the ponds were filled with filtered seawater (5 microns). Commercial feed with 35% of crude protein (Purina ®, Agribrands Purina Mexico, S.A. de C.V.) was used, the daily ration varied from 20 to 8% according to the shrimp weight. The feed was added each 2 h (12 times a day). In all treatments the carbon: nitrogen (C:N) ratio was of 12:1, to maintain it, different amounts of molasses were daily added as a carbon source.

2.4. Water quality monitoring

Every 6 h, dissolved oxygen and temperature were measured with an oximeter (YSI model 55; Yellow Spring, Ohio, USA); pH was measured twice a day with a potentiometer (Ohaus model ST 20; Parsippany, NJ, USA). Salinity was measured once a week, with a manual refractometer (Atago model PAL-SALT 4250; Minato, Tokyo, Japan).

Every three days, settleable solid (SS) were measured with Imhoff cones, the standardized sedimentation time was of 20 min. The total ammonia nitrogen (TAN), nitrite nitrogen (NO_2–N) and nitrate nitrogen (NO_3-N) were measured every week. For this purpose, water samples (250 mL) were collected and centrifuged, and the supernatant was used to measure the respective nutrients. The total ammonia nitrogen (TAN) was determined with the salicylate method (8155), NO_2-N by the diazotization with ferrous sulphate in acid medium method (8507) and