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Pacific white shrimp nursery trials in seawater and low-salinity water utilizing a synbiotic system

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Use of rice and wheat bran as organic carbon sources supports significant control of nitrogen compounds and enhanced production in *L. vannamei* nursery systems

This study assessed the performance of *L. vannamei* postlarvae in a synbiotic nursery system (seawater and low-salinity water) with rice bran and wheat bran as organic carbon sources. Results showed that this approach can provide significant control of nitrogen compounds (total ammonia nitrogen and nitrite-nitrogen) and help maintain water quality, as well as enhance the zootechnical performance of the nursed shrimp.

In Pacific white shrimp (*Litopenaeus vannamei*) intensive farming systems with minimal water exchange, the microbial community (heterotrophic and nitrifying bacteria) is stimulated by the organic carbon supply in the water that supports the transformation of nitrogenous compounds into less toxic compounds (i.e., nitrate) and also into microbial biomass. Several organic carbon sources have been used (including molasses, vegetable bran, dextrose and sugar) to stimulate bacterial growth. However, in recent years, new strategies like synbiotics have been incorporated into intensive aquaculture production, which are considered to have high potential for shrimp culture.

Synbiotic systems result from anaerobic and/or aerobic processes carried out by microorganisms (probiotics) on a vegetable or animal substrates, bran and other carbohydrates (prebiotics). Thus, probiotic microorganisms promote the breakdown of organic complex molecules into simpler molecules and provide balanced amounts of micronutrients and macronutrients to the animals in the aquaculture system. Moreover, there is substantial production of organic acids, such as lactic, acetic and butyric acids. This strategy provides a greater balance between the microorganisms and a lower supply of organic carbon in the system.

This article presents the results of a study that evaluated the effects of anaerobic and aerobic processes using rice bran and wheat bran as organic carbon sources (synbiotic) on the growth of *L. vannamei* postlarvae (PL) in seawater and low salinity water nursery systems.

Study setup

The studies were carried out at the Rural Federal University of Pernambuco, Recife, Brazil, with three trials with seawater and low salinity water. For the seawater trial, a water tank with a salinity of 35 grams per liter (g/L) was chlorinated with 13 mg/L of chlorine and dechlorinated by aeration for 72 hours. Then an inorganic fertilization treatment with urea (4.5 g N/m³), triple superphosphate (0.3 g P/m³) and sodium silicate (0.23 g Si/m³) was applied. In the low salinity water trial, a tank containing seawater diluted in freshwater to a salinity of 2 g/L was chlorinated with 13 mg/L of chlorine, and dechlorinated by aeration during 72 hours. Then, the organic fertilization was carried out as for the seawater trial.

L. vannamei postlarvae, 10 to 24 days old ($PL_{10\text{-}24}$), were stocked at a density of 2,000 to 3,000 shrimp/m³ in experimental units (40 and 60-L of useful volume). The PL were fed with a commercial shrimp feed (45 percent crude protein and 9.5 percent lipids) four times a day. The feeding rate was adjusted weekly based on shrimp growth, estimated consumption and mortality.

To evaluate the shrimp zootechnical performance, at the end of each trial, the animals were weighed to determine their final weight (g), feed conversion rate (FCR), yield (Kg/m³) and survival rate (percent).

Some water quality variables in the experimental units, such as dissolved oxygen (DO, mg/L), temperature (degrees-C) and salinity (g/L), were monitored daily. Other water quality variables like total ammonia nitrogen (TAN, mg/L), nitrite-N (NO_2^--N, mg/L) and alkalinity (mg $CaCO_3$/L) were measured weekly.

First trial

Organic fertilization was carried out with about 10 applications of the fertilizer. The fertilizer was processed through an anaerobic phase for 48 hours and an aerobic phase for 24 hours. During the experimental time, the fertilizer was added to the experimental units every three days during the 42 days of the trial. The organic fertilizer was composed of wheat bran (50 to 22.5 g/m³), molasses (25 to 12 g/m³) and sodium bicarbonate (10 to 4.5 g/m³). We also added (at 0.5 g/m³) a commercial (Kayros Ambiental and Agrícola, Brazil) bacteria-based product containing *Bacillus subtilis, B. licheniformis, Lactobacillus* sp., *Saccharomyces* sp. and *Pseudomonas* sp. strains at 7.7 x 10^8 CFU (colony forming units) per gram and previously chlorinated seawater.

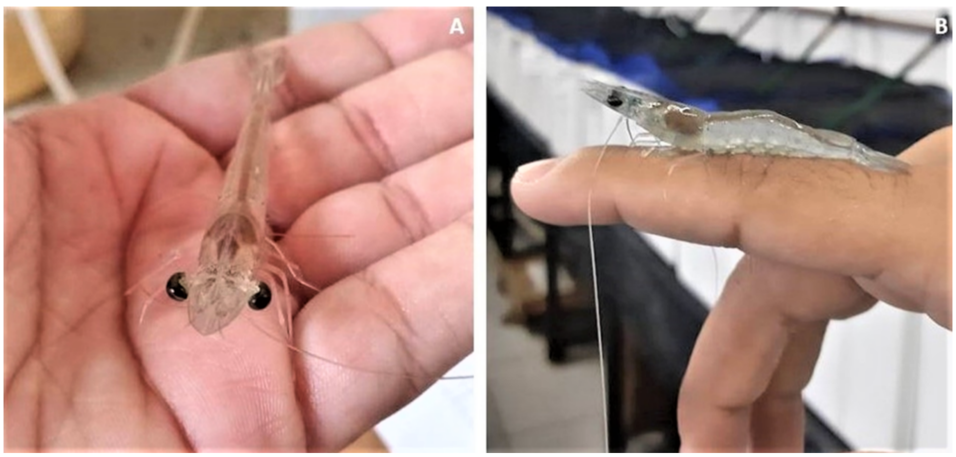
Second trial

Organic fertilization was carried out through about 10 applications of the fertilizer. The fertilizer was processed through an anaerobic phase for 24 hours and an aerobic phase for 24 hours. During the experimental time, the fertilizer was added to the experimental units every three days during the 42 days of the trial. The organic fertilizer was composed of rice bran (< 200 µm, 20 g/m³), molasses (2 g/m³), sodium bicarbonate (4 g/m³). We also added (at 0.5 g/m³) the commercial (Kayros Ambiental and Agrícola, Brazil) bacteria-based product containing *Bacillus subtilis, B. licheniformis, Lactobacillus* sp., *Saccharomyces* sp. and *Pseudomonas* sp. strains at 5.5 to 6.5 x 10^7 CFU per gram and previously chlorinated seawater.

Third trial

Organic fertilization was carried out through about 8 to 10 applications of the fertilizer. The fertilizer was processed through an anaerobic phase for 24 hours and an aerobic phase for 24 hours. During the experimental time, the fertilizer was added to the experimental units every three days during the 40 days of the trial. The organic fertilizer was composed by rice bran < 200 µm (20 g/m³), molasses (2 g/m³), sodium bicarbonate (4 g/m³).

We also added (at 0.5 g/m³) the commercial (Kayros Ambiental and Agrícola, Brazil) bacteria-based product containing *Bacillus subtilis, B. licheniformis, Lactobacillus* sp., *Saccharomyces* sp. and *Pseudomonas* sp. strains at 6.5×10^7 CFU per gram and previously chlorinated freshwater. In addition, in this trial and to support the microbial community development and the nitrification process in the experimental units, we added shells of the bivalve *Anomalocardia brasiliana* as artificial substrate (covering about 28 percent of the bottom (25 x 24 x 5 cm) area, and corresponding to 3.36 percent of the useful volume), in addition to reusing about 15 percent of the water in the shrimp nursery.



Harvested *L. vannamei* juveniles reared in the trials using a synbiotic nursery under seawater (A) and low salinity water (B) conditions.

Results and discussion

The water quality variables in the nurseries remained within the recommended range for intensive shrimp nurseries (Table 1). Mean values were ≈ 30.0 degrees-C for water temperature; DO levels over 5.0 mg/L; TAN levels of 0.33 to 0.60 mg/L; NO_2 – N levels of 0.41 to 1.56 mg/L; and alkalinity over 95 mg $CaCO_3$/L.

Brito, Nursery trials, Table 1

Variables	Trial 1	Trial 2	Trial 3
TAN (mg/L))	0.30 ± 0.11	0.60 ± 0.36	0.33 ± 0.54
NO2 – N (mg/L)	1.10 ± 0.49	1.56 ± 0.92	0.41 ± 0.13
Alkalinity (mg CaCO3/L)	118.34 ± 16.50	128.31 ± 3.08	95.47 ± 41.04

Table 1. Water quality data for the L. vannamei nursery trials in seawater and low salinity water conditions using a synbiotic system fertilized with wheat and rice bran processed by anaerobic and aerobic phases. Trial data are the mean ± standard deviation. TAN: total ammonia nitrogen; NO2 - N: nitrite-nitrogen.

The shrimp zootechnical performance data in the synbiotic nursery with seawater and low salinity water conditions at days 40-42 are presented in Table 2. Survival rates were over 84 percent, final shrimp weights were 0.85 – 0.98 grams, and mean feed conversion rates were 1.20 – 1.34 while reaching a shrimp yield of 1.53 – 2.50 Kg/m³ (Table 2).

Brito, Nursery trials, Table 2

Variables	Trial 1	Trial 2	Trial 3
Final weight (g)	0.98 ± 0.16	0.96 ± 0.31	0.85 ± 0.37
Yield (Kg/m3)	2.48 ± 0.03	2.50 ± 0.86	1.53 ± 0.71
Survival (%)	84.45 ± 10.54	92.54 ± 4.47	89.01 ± 5.60
FCR	1.28 ± 0.27	1.20 ± 0.26	1.34 ± 0.50

Table 2. Performance of the L. vannamei postlarvae grown in seawater and low salinity water conditions using a synbiotic system fertilized with wheat and rice bran processed by anaerobic and aerobic phases. Trial data are the mean ± standard deviation. FCR: feed conversion rate.

Based on our study results, we showed that the combination of anaerobic and aerobic processing of wheat bran and rice bran by microorganisms and their use as an organic carbon source, and also the use of artificial substrate in *L. vannamei* nurseries are a viable alternative for synbiotic system fertilization using minimal water exchange. This approach allows greater control of total ammonia nitrogen and nitrite-nitrogen in seawater and low salinity water systems, unlike what happens in intensive systems with minimal water exchange with low salinity, which have shown high mortality to the cultured shrimp due to the toxicity of these nitrogen compounds.

Overall, our results demonstrated that the use of the water fertilization method we used contributes positively to the growth of beneficial heterotrophic and nitrifying bacteria and thus supports improved shrimp growth and survival in seawater and low-salinity water conditions.

Perspectives

The results of our study show that the combined administration of vegetable bran (anaerobic and aerobic) as an organic carbon source and probiotics (synbiotic), artificial substrate and water reuse provides significant control of nitrogen compounds (TAN and NO_2^--N) and positive effects on the growth of *Litopenaeus vannamei* in nurseries with seawater and low-salinity water.

References available from the corresponding author.

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