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Utilization of rice byproducts as carbon sources in high-density culture of the Pacific white shrimp, *Litopenaeus vannamei*

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ABSTRACT - This study was conducted to evaluate the effect of rice byproducts on water quality, microbial community, and growth performance of *L. vannamei* juveniles. Shrimp of 0.98±0.10 g body weight (BW) were reared in 49 tanks of 1.5 m^3 under 127 animals m^{-2} for 77 days. Rice bran, rice grits, and rice hulls were mixed into five different fertilizers varying their fiber content (90, 110, 150, 200, and 250 g kg^{-1}) and compared against sugarcane molasses (MO) and unfertilized tanks (UNF). Rice byproducts and MO were applied in water three times a week at a fixed rate of 4.5 g m^{-3}. Water salinity, pH, temperature, and dissolved oxygen reached 43±2 g L^{-1}, 8.03±0.32, 30.2±0.90 °C, and 5.03±0.53 mg L^{-1}, respectively. Settleable solids (SS) were higher in tanks fertilized with rice byproducts (from 2.5±1.0 to 3.1±1.1 mL L^{-1}) and MO (3.4±1.0 mL L^{-1}). Total ammonia nitrogen (0.19±0.09 mg L^{-1}), nitrite (5.97±2.04 mg L^{-1}), and nitrate (1.29±0.48 mg L^{-1}) were kept low without any significant differences among treatments. The concentration of heterotrophic bacteria and fungi was significantly higher in rice byproducts compared with MO. Water fertilization had no effect on final shrimp survival (85.5±9.5%), weekly growth (0.72±0.11 g), and feed conversion ratio (1.59±0.10). Tanks treated with rice byproducts, except with 90 g kg^{-1} fiber, resulted in a higher final shrimp BW (from 9.04±1.56 to 9.52±1.89 g) compared with MO (8.75±2.14 g) and UNF (7.74±1.48 g). Gained yield and feed intake were significantly higher for tanks treated with rice byproducts than with UNF. A mix of rice byproducts can be equally or more effective as carbon sources to shrimp culture than MO.

Keywords: microbial community, organic fertilization, shrimp growth performance

Introduction

Marine shrimp aquaculture requires new technologies to eliminate and control water exchange, discharge of effluents, disease outbreaks, and overuse of feeds (Lara et al., 2012). In recent years, high-density shrimp farming under limited water exchange has been possible through manipulation of microbial communities in water (Azim and Little, 2008; Samocha et al., 2010; Krummenauer et al., 2011; Audelo-Naranjo et al., 2012). The principle of minimum water exchange crops is based on the addition of carbon sources to balance the C:N ratio in water. This promotes the growth of microorganisms that consume organic matter, improve nutrient utilization, and convert dissolved nitrogen into less toxic compounds (Avnimelech, 2007; Emerenciano et al., 2013).

Several sources of carbon have been used for this purpose, including sugarcane molasses, glycerol, vegetable sugar, soybean meal, wheat flour, wheat bran, maize bran, rice bran, and tapioca flour (Hari et al., 2004; Wang et al., 2016; Ekasari et al., 2014; Romano et al., 2018). They are chosen according to cost, local availability, biodegradability, and assimilation efficiency by microorganisms (Emerenciano et al., 2013).

World rice production in 2018 was estimated at 773 million MT, of which 513 million MT were processed (FAO, 2018). Rice is commonly produced by removing the hull and bran layers of the rough rice kernel in hulling and milling processes, respectively (Saman et al., 2019). Rice bran, rice grits, and rice hulls are the main rice byproducts (Lorenzett et al., 2012). They can contain 40% carbohydrates and moderate levels of crude protein (12%) and lipids (21%) (Lima et al., 2000; Vilani et al., 2016).

Rice hulls account for approximately 20% by weight of the seeds, generating millions of MT of waste every year (Stracke et al., 2018). These residues, if not disposed properly, are sources of environmental pollution as they are difficult to degrade (Saidelles et al., 2012). Studies have shown that rice residues can be used to improve shrimp and fish culture. Serra et al. (2015) found that *L. vannamei* performs better when water is fertilized with rice bran compared with dextrose. Similarly, Vilani et al. (2016) reported that in tanks fertilized with rice bran, juvenile *L. vannamei* achieves an increased yield and lower feed conversion ratio (FCR) compared with tanks treated with sugarcane molasses. Romano et al. (2018) used rice bran for the rearing of African catfish juveniles (*Clarias gariepinus*) and observed a significant increase in fish growth and feed efficiency.

This study evaluated the effect of using different combinations of rice byproducts (rice bran, rice grits, and rice hulls) as carbon sources on water quality, microbial community, and growth performance of juveniles of the whiteleg shrimp (*Litopenaeus vannamei*) reared under limited water exchange.

Material and Methods

Rice byproducts (rice bran, rice grits, and rice hulls) were obtained from a rice processing industry (Sucesso Agroindústria Ltda., Eusébio, Brazil), cultivars IRGA 424 and PUITÁ INTA-CL. Their proximate composition was determined according to the Brazilian compendium of animal feeding (Table 1, SINDIRAÇÕES, 2013).

Five fertilizer mixtures with different concentrations of rice bran, rice grits, and rice hulls were designed (Table 2). Fertilizers were formulated to present a nearly similar value of total carbon with a gradual increase in their crude fiber content. This maximized the use of rice hulls, which have the lowest economic value among these byproducts. Fertilizers were identified according to their crude fiber concentration (F90, F110, F150, F200, and F250). The F90 mixture was composed of 50% rice grits, 40% rice bran, and 10% rice hulls (as is basis). The progressive increase in crude fiber was achieved by consecutive replacements of rice bran for rice hulls at 25% each.

Table 1 - Chemical composition (g kg^{-1}, dry matter) of rice byproducts used in the preparation of fertilizers

Composition (g kg^{-1})	Rice bran	Rice grits	Rice hulls
Dry matter	901.2	871.3	897.5
Crude protein	150.8	92.4	23.1
Lipids	147.9	13.7	11.3
Crude fiber	85.1	6.7	568.1
Nitrogen	24.1	14.8	3.7
Calcium	0.7	0.2	1.0
Phosphorous	15.0	2.6	0.1
Potassium	11.3	2.0	2.2
Ash	87.7	12.6	88.0
Insoluble residues	26.1	2.8	81.2
Total carbohydrates	528.5	874.7	309.5
Total carbon	418	405	370

Table 2 - Chemical composition (g kg^{-1}, dry matter) and texture of rice byproduct fertilizers and sugarcane molasses (MO)

Item	Fertilizer					
	F90	F110	F150	F200	F250	MO
Composition[1] (g kg^{-1})						
Dry matter	888.5	896.2	896.7	897.0	898.1	930.3
Crude protein	105.1	115.7	96.2	80.8	70.0	36.3
Lipids	64.0	85.5	49.2	32.3	17.3	14.0
Crude fiber	92.2	110.2	147.0	203.3	248.9	1.3
Nitrogen	16.9	18.5	15.4	12.9	11.2	5.8
Calcium	1.0	1.3	1.3	0.6	0.6	62.1
Phosphorous	0.7	0.9	0.6	0.4	0.3	0.5
Potassium	5.5	7.1	5.1	4.2	3.6	29.2
Ash	50.8	62.7	47.8	46.9	50.8	210.9
Insoluble residues	17.6	23.8	25.1	26.8	36.5	9.7
Total carbohydrates	687.9	625.9	659.8	636.6	613.1	737.5
Total carbon	405	408	401	396	389	322
C:N ratio	24	22	26	31	35	55
Mesh (µm)	% Retained[2]					
1.000	0.02	0.09	0.01	0.01	0.08	-
850	0.05	0.13	0.05	0.05	0.07	-
600	0.84	1.74	1.44	1.90	2.49	-
425	3.28	6.90	7.01	7.64	8.38	-
300	38.62	49.29	31.95	18.42	18.38	-
250	33.90	29.45	37.50	35.29	21.77	-
< 250	23.30	12.41	22.04	36.69	48.77	-

[1] Analysis according to the standards of the Brazilian compendium of animal feeding (SINDIRAÇÕES, 2013).
[2] Determined on a sieve shaker (MA750, Marconi Equipamentos para Laboratórios Ltda, Piracicaba, São Paulo, Brazil).

To prepare the fertilizers, byproducts were first ground through a 500-µm mesh in a hammer mill (MCS 280, Moinhos Vieira, Tatuí, Brazil) and then mixed for 10 min with a planetary mixer (AR 25, G. Paniz, Caxias do Sul, Brazil). More than 85% of the total composition of the fertilizers was less than 300 µm, therefore, physically characterized as powder (Brasil, 2016). The processed fertilizers showed a concentration of insoluble residues directly proportional to their crude fiber content (Table 2).

Dried sugarcane molasses (Indumel - Industria e Comércio de Melaço Ltda., Sertãozinho, Brazil) were used as a positive control (MO) as it has been shown to act as an efficient carbon source for shrimp culture (Samocha et al., 2007; Krummenauer et al., 2011; Schveitzer et al., 2013; Arantes et al., 2017; Espírito Santo et al., 2017). Seven tanks without any direct application of carbon sources acted as a negative control (UNF).

The study was carried out in 49 independent outdoor tanks of 1.5 m^3 (1.61 m^2 of bottom area, 0.83 m height, with 1.43 and 1.75 m of bottom and surface diameter, respectively). Each tank was equipped with an individual water inlet and outlet. Supplemental aeration was carried out with one 7.5-hp blower connected to a flexible 0.50-m micro-perforated hose kept individually in each tank bottom.

The system operated in a static condition, with limited water exchange. Seawater was supplied biweekly to compensate for evaporative losses and increase in water salinity. Levels of settleable solids (SS) and total suspended solids (TSS) were kept at 10-14 mL L^{-1} and between 250 and 350 mg L^{-1} (Samocha et al., 2017), respectively. Water exchange was only carried out twice during culture, at 5% of total water volume, when SS and TSS ranges were exceeded. Thus, nitrogen accumulation was reduced through water exchange in both fertilized and unfertilized tanks.

To prepare culture water, rearing tanks were initially filled with filtered seawater (salinity 32 g L^{-1}) and inoculated with 100 L of water obtained from a shrimp nursery tank. For initial water fertilization, 10 g m^{-3} of ground shrimp feed (Camanutri 35, Neovia Nutrição e Saúde Animal Ltda., São Lourenço da Mata, Brazil) and 4.5 g m^{-3} of each fertilizer were applied daily to each tank at a carbon to nitrogen (C:N) ratio near 10:1 (Avnimelech, 1999). This application occurred for five consecutive days. To sustain the medium during shrimp culture, rice byproducts and molasses were applied in water three times a week during the complete rearing period. Fertilizers were applied at the same fixed rate (4.5 g m^{-3}) provided that the SS did not exceed the limit of 14 mL L^{-1} as established by Samocha et al. (2017).

Shrimp of 0.98±0.10 g (mean ± standard deviation; n = 9,996) were stocked under 127 animals m^{-2} (204 shrimp $tank^{-1}$). They were fed daily, 10 times a day with an automatic feeder (described in Nunes et al., 2019) that operated between 07.00 and 17.00 h. Animals were fed a grower commercial shrimp feed containing a minimum of 38% crude protein (Density 38, Neovia Saúde e Nutrição Animal Ltda., São Lourenço da Mata, Brazil). Meals were adjusted daily following an estimated weight gain of 100 mg day $shrimp^{-1}$ and an estimated 0.5% weekly drop in shrimp survival. Biweekly (days 15, 30, 45, and 60 of rearing), meals were adjusted by individually weighing ten animals per tank. Feeding rates were calculated based on the maximum amount of feed (MM, g) that can be eaten daily by one individual of a specific body weight (BW), in accordance with the formula $MM = 0.0931BW^{0.6200}$ (Nunes and Parsons 2000; Nunes et al., 2006; Façanha et al., 2018). To avoid excess feeding and a high FCR, feeding rates were reduced by 30% across all diets (Nunes et al., 2006). All rearing procedures were performed in compliance with relevant laws and institutional guidelines, including those related to animal welfare.

Water salinity, pH, temperature, and dissolved oxygen (DO) were measured daily in each tank, reaching a mean (± standard deviation) of 43±2 g L^{-1} (n = 3,067), 8.03±0.32 (n = 3,066), 30.2±0.90 °C (n = 3,066), and 5.03±0.53 mg L^{-1} (n = 3,036), respectively. These parameters fell within the limits tolerated by *L. vannamei* juveniles (Wyk, 1999), including water salinity (Castro et al., 2018). No statistical differences were observed in these parameters between treatments (P>0.05).

Total ammonia nitrogen (TAN), nitrite (NO_2^-), and nitrate (NO_3^-) concentrations were determined weekly in two pools of water sampled from each treatment (n = 140) using a mass spectrophotometer (DR 2800 Spectrophotometer, Hach Company, Loveland, USA). Alkalinity and TSS determinations were performed biweekly (APHA, 2012). Settleable solids were measured every two days with Imhoff cones (APHA, 2012).

Shrimp were harvested after 77 days of culture. All animals were counted and weighed individually to determine final survival (%), body weight (g), weekly growth (g), and gained yield (g m^{-2}). Feed conversion ratio and apparent feed intake (AFI, g of feed delivered divided by the number of stocked shrimp) were calculated in an as is basis.

Microbiological analyzes were performed on fertilizers. These analyses followed the standard plate count (SPC) for determination of the concentration of heterotrophic bacteria (HB), *Bacillus* spp., fungi, and *Vibrio* spp. present in each fertilizer. For these analyzes, 10 g of each fertilizer were diluted in 90 mL of 10 g L^{-1} saline solution with serial dilutions of 10^{-2} to 10^{-5}. For the quantification of HB, an aliquot of 0.01 mL was used by the plating method in depth using Plate Count Agar medium (7157A, Acumedia, Neogen, Indaiatuba, Brazil). Isolation of *Bacillus* spp was performed by carrying out a water bath at 70 °C for 1 h – 30 min longer than recommended by the method (Pandey et al., 2013). A heat shock until sporulation, for the quantification an aliquot of 0.01 mL, was used by the plating method in depth using Plate Count Agar medium (7157A, Acumedia, Neogen, Indaiatuba, Brazil). Plates were read after 48 h of incubation at 35 °C.

To quantify the fungi, the spread plate technique was used, in which an aliquot of 100 μL of the respective dilutions (10^{-2} to 10^{-5}) were added in Petri dishes containing the solidified medium of Potato

Dextrose Agar (Himedia, Mumbai, India), plus 10 μL mL^{-1} ampicillin and 1.8% tartaric acid solution. Subsequently, the plates were incubated at 28 °C for up to seven days. For the analysis of *Vibrio* spp., the medium used was Thiosulfate Citrate Bile Saccharose Agar (7210, Acumedia, Neogen, Indaiatuba, Brazil) with the spread plate technique and incubation at 35 °C for 18 h.

After the incubation period of all analyzes, plaques between 25 and 250 colonies were counted. Plates outside this interval were estimated. For the calculation of SPC, the following equation was applied: SPC = cfu (colony forming unit) × the inverse of the dilution factor × correction factor (Downes and Ito, 2001).

The effect of organic fertilizers on water quality (TAN, NO_2^-, NO_3^-, SS, TSS, and alkalinity) and shrimp growth performance parameters (final survival, final body weight, growth, gained yield, FCR, and AFI) were analyzed using One-Way ANOVA. The following mathematical model was adopted:

$$Yij = \mu + \tau i + \epsilon ij, \tag{1}$$

in which Yij is the *j*-th observation of fertilizer i; μ is the general mean response; τi is the non-random effect of fertilizers, in which $\sum_{i=1}^{k} \tau i = 0$; and ϵij is the random fertilizer error. When significant differences were detected, they were compared two-by-two with Tukey's HSD. The significance level of 5% was applied in all statistical analyses. Statistical package SPSS 15.0 for Windows was used (SPSS Inc., Chicago, Illinois, United States).

Results

Shrimp reached mean (± SD) final survival, weekly growth, and FCR of 85.5±9.5%, 0.72±0.11 g, and 1.59±0.10, respectively (Table 3). No significant responses on these variables could be associated with the organic carbon sources (P>0.05). However, gained yield (g m^{-2}) was significantly higher in treatments fertilized with rice byproducts (F110, F150, F200, and F250) compared with the unfertilized treatment (UNF) (P<0.05). Likewise, a higher AFI was observed in tanks treated with fertilizers produced with rice byproducts compared with the UNF. There was no difference in AFI between MO and UNF (P>0.05).

The SS concentration varied during culture in all treatments (Figure 1). There was a progressive increase in SS up to the 27th day of culture when a water exchange was performed. Thereafter, the upward trend was maintained, controlled again on the 55th day by a new water exchange. There was no significant difference in TSS (485±74 mg L^{-1}, n = 49) and alkalinity (172±27 mg $CaCO_3$ L^{-1}, n = 42) among the experimental treatments.

The concentration of TAN (0.19±0.09 mg L^{-1}), nitrite (5.97±2.04 mg L^{-1}), and nitrate (1.29±0.48 mg L^{-1}) was not different among treatments (P>0.05). However, there was a significant difference in the concentration of nitrogenous compounds (P<0.05) among the initial (1st-28th days), intermediate (29th-46th days), and final (47th-64th days) culture phases. In the final phase, TAN concentration was higher (0.27±0.09 mg L^{-1}) compared with the initial (0.17±0.06 mg L^{-1}) and intermediate

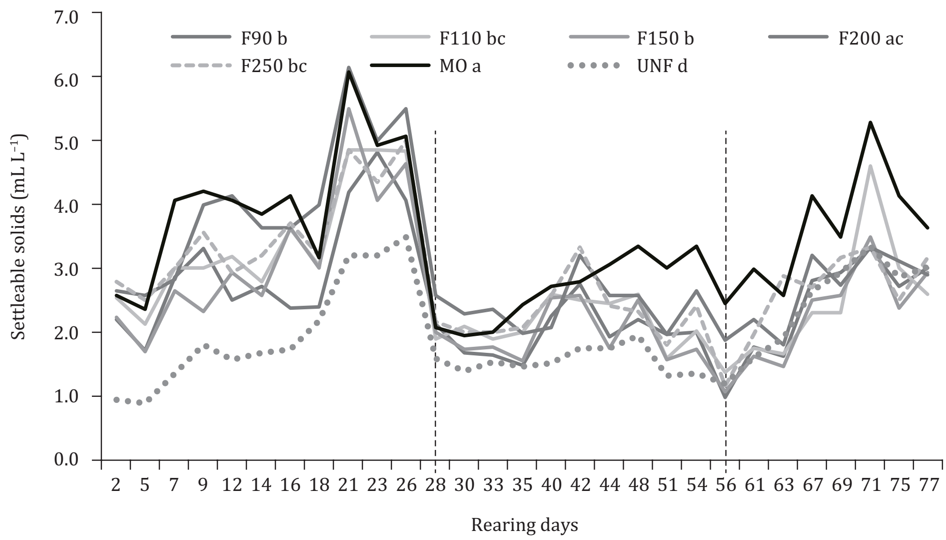
Table 3 - Growth performance of *L. vannamei* (values refer to the mean ± standard deviation of seven culture tanks)

Fertilizer	Final survival (%)	Growth (g $week^{-1}$)	Final body weight (g)	Gained yield (g m^{-2})	FCR	AFI (g $shrimp^{-1}$)
F90	88.1±5.5a	0.71±0.06a	8.78±1.52d	810±69ab	1.55±0.10a	10.5±0.3a
F110	87.3±8.2a	0.75±0.13a	9.17±1.95bc	842±62a	1.55±0.06a	10.8±0.6a
F150	82.1±13.7a	0.77±0.13a	9.32±2.04ab	826±38a	1.56±0.03a	10.7±0.4a
F200	83.2±9.7a	0.79±0.11a	9.52±1.89a	821±51a	1.56±0.04a	10.7±0.6a
F250	88.1±3.9a	0.73±0.06a	9.04±1.56c	827±56a	1.55±0.07a	10.6±0.3a
MO	81.3±14.2a	0.73±0.16a	8.75±2.14d	736±76ab	1.67±0.15a	10.2±0.6ab
UNF	88.7±8.3a	0.62±0.06a	7.74±1.48e	706±63b	1.63±0.13a	9.6±0.4b

FCR - feed conversion ratio; AFI - apparent feed intake; MO - dried sugarcane molasses (positive control); UNF - unfertilized tanks (negative control).
Different letters in the same column indicate statistical difference (P<0.05) according to Tukey's HSD test.

(0.06±0.14 mg L^{-1}) phases. Comparatively, nitrite and nitrate showed statistically lower concentrations before shrimp harvest (5.36±2.34 and 1.24±0.37 mg L^{-1}, respectively) compared with initial (6.91±1.70 and 1.62±0.47 mg L^{-1}, respectively) and intermediate (5.89±1.80 and 1.11±0.49 mg L^{-1}, respectively) phases.

Fertilizers F90, F110, F150, and F250 showed a significantly higher concentration of HB compared with the MO and UNF treatments (Table 4). *Bacillus* spp. were more concentrated in the MO (9.30±1.10 × 10^4 cfu mL^{-1}) than in other treatments (P<0.05). The concentration of fungi was higher under rice byproduct treatments with a higher fiber level (F200 and F250). The only fertilizer with *Vibrio* spp. was F110 (0.004 ± <0.001 × 10^4 cfu mL^{-1}).



MO - dried sugarcane molasses (positive control); UNF - unfertilized tanks (negative control).
Vertical lines indicate water exchange.
Different letters in the legend represent statistical difference in SS (P<0.05) between fertilizers according to the Tukey's HSD test.

Figure 1 - Variation of settleable solids concentration in cultured water treated with different fertilizers over 77 days.

Table 4 - Concentration (10^4 cfu mL^{-1}) of heterotrophic bacteria (HB), *Bacillus* spp., fungi, and *Vibrio* spp. in carbon sources

Fertilizer	Standard plate count (10^4 cfu mL^{-1})			
	HB	*Bacillus* spp.	Fungi	*Vibrio* spp.
F90	245.0±7.1a	5.20±0.70c	0.96±0.33ab	<0.001b
F110	161.5±61.5ab	7.40±0.71b	0.79±0.13ab	0.004±<0.001a
F150	227.0±28.3a	0.83±0.01d	0.52±0.04bc	<0.0001b
F200	98.0±32.5b	0.84±0.02d	1.27±0.04a	<0.0001b
F250	165.0±22.6ab	1.60±0.26d	1.10±<0.01a	<0.0001b
MO	0.01±<0.01c	9.30±1.14a	<0.01c	<0.0001b
UNF	1.65±0.2c	0.34±0.06d	<0.001±<0.01c	<0.0001b

MO - dried sugarcane molasses (positive control); UNF - unfertilized tanks (negative control).
Different letters in the same column indicate statistical difference (P<0.05) according to Tukey's HSD test.
Each value represents the reading (mean ± standard deviation) of two samples at five dilutions.

Discussion

Results demonstrated that a mix of rice byproducts can be equally or more effective as carbon sources to shrimp culture than sugarcane molasses. Shrimp final BW and gained yield, apparent feed intake, and water quality parameters were similar or higher under treatments subjected to fertilization with rice byproducts compared with molasses. It is likely that rice byproducts were also used as a food source by shrimp, either directly or indirectly. Rice byproducts contain higher levels of crude protein (70.0 to 115.7 g kg^{-1}) and lipids (17.3 to 85.5 g kg^{-1}) than molasses (36.3 and 14 g kg^{-1}, respectively). Serra et al. (2015) working with *L. vannamei* post-larvae and juveniles reported a better growth performance in tanks fertilized with rice bran compared with molasses, because shrimp consumed the former directly.

One of the possible deleterious effects associated with the use of rice byproducts is the presence of a relatively high crude fiber content (Romano et al., 2018). Fiber is considered to be difficult to metabolize by microorganisms and shrimp, and accumulation in the culture environment may take place. However, it was possible to demonstrate that the application of carbon sources using high concentrations of rice hulls, which are the most discarded rice byproduct, resulted in a higher final shrimp BW and an increased gained yield compared with UNF. This suggests that rice hulls may assist in microbial colonization, resulting in an improved shrimp performance. Therefore, crude fiber concentrations of up to 200 g kg^{-1} with three weekly application rates of 4.5 g m^{-3} did not generate negative effects on water quality and shrimp performance.

These results corroborate the study by Ekasari et al. (2014). The authors compared the use of rice bran, tapioca flour, tapioca byproduct, and sugarcane molasses as fertilizers in the culture of *L. vannamei* juveniles. The crude fiber levels in rice bran and tapioca byproduct reached 133 and 79 g kg^{-1}, respectively. No negative effects were associated with these levels of fiber. In fact, authors reported a better shrimp survival and protein assimilation with rice bran and tapioca byproduct than with molasses.

The minimum water exchange and the high shrimp density increased the amount of organic matter in culture water, which favors the development of *Vibrio* spp. (Ferreira et al., 2011). Although *Vibrio* spp. is part of the natural microbiota of shrimp, some 70 strains of *V. harveyi* and *V. parahaemolyticus* have been known to cause serious shrimp outbreaks (Tran et al., 2013). However, the concentration of *Vibrio* spp. in fertilizers was below levels reported during vibriose outbreaks, *i.e.*, $>1 \times 10^4$ cfu mL^{-1} (Soto-Rodriguez et al., 2015). It has been demonstrated that the bacterial community established in super-intensive culture systems with fertilizers can inhibit the proliferation of pathogens by competitive exclusion (Crab et al., 2010).

It was observed that fertilizers made from rice byproducts showed a higher concentration of HB, *Bacillus* spp., and fungi compared with the UNF. This may have benefited shrimp performance through their direct ingestion. These microorganisms utilize a diverse range of carbon sources from agriculture for their growth (Thomsen, 2005). They are able to produce endogenous enzymes in the shrimp hepatopancreas (Anand et al., 2014; Panigrahi et al., 2019), likely resulting in a greater nutrient availability and improved shrimp performance.

Conclusions

A mix of rice byproducts can effectively act as carbon sources in shrimp farming, promoting the development of bioflocs and improving shrimp performance. Crude fiber in rice byproducts as high as 200 g kg^{-1} has no detrimental effect to shrimp survival and growth and water quality when applied three times a week at 4.5 g m^{-3}. Thus, it is possible to grow *L. vannamei* juveniles in intensive culture under minimum water using a mix of rice byproducts to maintain water quality standards and increase shrimp growth performance.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: A.J.P. Nunes. Data curation: J.S. Leite, C.S.B. Melo and A.J.P. Nunes. Formal analysis: J.S. Leite and A.J.P. Nunes. Funding acquisition: J.S. Leite and A.J.P. Nunes. Investigation: J.S. Leite, C.S.B. Melo and A.J.P. Nunes. Methodology: J.S. Leite and A.J.P. Nunes. Project administration: J.S. Leite and A.J.P. Nunes. Resources: C.S.B. Melo. Supervision: A.J.P. Nunes. Writing-original draft: J.S. Leite. Writing-review & editing: A.J.P. Nunes.

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