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PERFORMANCE OF PACIFIC WHITE SHRIMP FED PROBIOTICS *Lactobacillus plantarum* AND *Bacillus* spp. IN A BIOFLOC SYSTEM*

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ABSTRACT

The present study evaluated the use of the indigenous probiotic *Lactobacillus plantarum* and commercial probiotic containing *Bacillus* spp. in the culture of *Litopenaeus vannamei* in a biofloc system. Shrimp were fed four diets: *L. plantarum*, *Bacillus* spp., *L. plantarum* + *Bacillus* spp. and feed with no additives. Growth performance, water quality variables, microbiological counting of water and digestive tract of shrimp were determined. The control group and *L. plantarum* treatment showed better growth performance. The highest feed conversion ratio (FCR) and the lowest survival were obtained in the *L. plantarum* + *Bacillus* spp. treatment, which had significantly higher nitrite values. *Vibrio* spp. counts in the water were lower in the *L. plantarum* and *L. plantarum* + *Bacillus* spp. treatments and were lower in the intestinal tract in the *L. plantarum* treatment. Lactic acid bacteria (LAB) was higher in the *L. plantarum* treatment in the water and digestive tract. The count of total heterotrophic bacteria (THB) deferred only among *Bacillus* spp. and *L. plantarum* + *Bacillus* spp. treatment, being higher in the latter group. In *Bacillus* spp. treatment, no presence of LAB was detected in the water or intestinal tract. We conclude that the use of *L. plantarum* combined with *Bacillus* spp. negatively affected survival, FCR and water quality, but that the use of *L. plantarum* alone reduced the presence of *Vibrio* spp., even though it did not change the growth performance of *L. vannamei*.

Key words: *Litopenaeus vannamei*; *Vibrio* spp.; BFT; microbiology.

DESEMPENHO DO CAMARÃO BRANCO PACÍFICO ALIMENTADO COM PROBIÓTICO *Lactobacillus plantarum* E *Bacillus* spp. EM SISTEMA BIOFLOCOS

RESUMO

O presente estudo avaliou o uso do probiótico endógeno *Lactobacillus plantarum* e do probiótico comercial contendo *Bacillus* spp. no cultivo de *Litopenaeus vannamei* em sistema de bioflocos. Camarões foram alimentados com quatro dietas: *L. plantarum*, *Bacillus* spp., *L. plantarum* + *Bacillus* spp. e ração sem aditivos. Foram avaliados o crescimento, a qualidade da água e contagem microbiológica na água e intestino dos camarões. O controle e o tratamento com *L. plantarum* apresentaram melhor desempenho de crescimento. O maior fator de conversão alimentar (FCA) e a menor sobrevivência foram obtidas no tratamento *L. plantarum* + *Bacillus* spp., apresentando valores de nitrito significativamente elevados. As contagens de *Vibrio* spp na água foram menores nos tratamentos *L. plantarum* e *L. plantarum* + *Bacillus* spp. e igualmente foram menores no intestino no tratamento com *L. plantarum*. As contagens de bactérias ácido lácticas (BAL) foram maiores no tratamento *L. plantarum* na água e no trato intestinal. A contagem de bactérias heterotróficas totais (BHT) diferiu apenas entre os tratamentos *Bacillus* spp. e *L. plantarum* + *Bacillus* spp., sendo maior no último grupo. No tratamento *Bacillus* spp. não foi detectada presença de BAL na água nem no trato intestinal. Concluímos que o uso de *L. plantarum* combinado com *Bacillus* spp. afetou negativamente a sobrevivência, o FCA e a qualidade da água, contudo o uso de *L. plantarum* isoladamente reduziu a presença de *Vibrio* spp., embora não tenha alterado o desempenho de crescimento de *L. vannamei*.

Palavras-chave: *Litopenaeus vannamei*; *Vibrio* spp.; BFT; microbiologia.

INTRODUCTION

In recent years, intensification associated with the failure to adopt good production practices has been responsible for the emergence of both viral and bacterial diseases, causing important economic losses in marine shrimp farming (FAO, 2020). To reduce this problem, more efficient and sustainable alternative technologies, such as biofloc technology (BFT), have been adopted (Krummenauer et al., 2014). BFT allows production at high densities, maintaining water quality and improving the growth and survival of animals through the balance of microbial communities in the system without water exchange, consequently improving biosecurity (Wasielesky et al., 2006; Emerenciano et al., 2017).

Although BFT is a production system that encourages the appearance of a microbial community that can help inhibit pathogens, the biofloc system can be vulnerable to outbreaks of harmful organisms. Bacteria of the genus *Vibrio* are considered opportunistic or secondary pathogens and are naturally present in the shrimp farming environment and intestinal microbiota (Gopal et al., 2005). This pathogen is highly capable of taking advantage of changes in the system and occupying ecological niches related to the use of water as a growing environment in aquaculture systems. (Skjermo and Vadstein, 1999).

To maintain balance in the system and reduce the presence of potentially pathogenic bacteria, BFT has begun to use probiotic microorganisms. Probiotics are beneficial living microorganisms capable of improving host health, as well as conferring beneficial effects on the microbial community of the system (Verschuere et al., 2020). These probiotics can be indigenous bacteria with a better adaptation, reducing the possible imbalances that non-indigenous bacteria may generate (Gullian et al., 2004; Knipe et al., 2021). In aquaculture farms using the biofloc system, commercial bacteria consortia are commonly used as probiotics (Emerenciano et al., 2017; Arias-Moscoso et al., 2018). These commercial probiotics have gained more interest in aquaculture since the use of several strains together may have a synergistic effect by the wide range of probiotic activity (Merrifield et al., 2010; Nayak, 2010; Ringø, 2020).

However, aquaculture farmers are generally uncertain about the effect of many different types of probiotics marketed and their frequent indiscriminate and incorrect use in different species and environmental conditions (Uddin et al., 2015). This failure to recognize the importance of selectivity can have potentially negative consequences, causing imbalance and damage to both cultivated species and the natural environment (Vargas-Albores et al., 2017). It is important to note that unsuccessful results obtained in many studies carried out with probiotics are attributed to inadequate selection of microorganisms (Lazado et al., 2015). Therefore, in selecting a probiotic, it is important to evaluate beforehand whether the probiotic to be used will be commercial, isolated from the host, isolated from another species, or if a single microorganism or consortium will be used, among other factors (Ringø, 2020), because not all probiotics may adapt to all species and/or environmental conditions.

Therefore, the aim of this study was to evaluate the individual and combined use of the indigenous probiotic *L. plantarum* and a consortium of commercial probiotics containing *Bacillus* spp. in the marine shrimp *Litopenaeus vannamei* cultivated in a biofloc system.

MATERIAL AND METHODS

The experiment was conducted at the Laboratório de Camarões Marinhos (LCM) at the Universidad Federal de Santa Catarina (UFSC).

Biological material

Shrimp juveniles (HB16-Aquatec LTDA), with an average weight of 2.00 ± 0.02 g, were obtained from an LCM biofloc rearing tank.

The probiotic bacteria used were *Lactobacillus plantarum* (CPQBA 007 07 DRM01) isolated from the intestinal tract of *L. vannamei* (Vieira et al., 2008) and a commercial probiotic composed of *Bacillus* spp. (*Bacillus subtilis, Bacillus licheniformis* and *Bacillus pumilus*).

Experimental design

Shrimp were distributed in 12 round fiberglass tanks, with a 9000 L volume of water, provided with aeration (aerotubes) and heaters coupled with thermostats. Before the stocking process, the experimental units were inoculated with 30% water of mature biofloc from the laboratory with the following characteristics: ammonia, 0.3 ± 0.1 mg L^{-1}; nitrite, 0.2 ± 0.0 mg L^{-1}; nitrate, 6.5 ± 0.8 mg L^{-1}; alkalinity, 131.3 ± 4.4 mg L^{-1} $CaCO_3$; pH, 7.4; salinity, 34.8; orthophosphate, 1.0 ± 0.0 mg L^{-1}; total suspended solids, 217.9 ± 44.2 mg L^{-1}; volatile, 31.7 ± 3.0%; and fixed, 68.3 ± 3.0%. Every unit was stocked with shrimp at a density of 300 shrimp m^{-3} (2700 shrimp $tank^{-1}$) and divided into four treatments, with three replicates each, in a completely randomized design.

Treatments

The experiment was carried out with four different diets: 1) commercial feed with probiotic *L. plantarum* (10^7 CFU mL^{-1} of *L. plantarum*); 2) commercial feed with probiotic *Bacillus* spp. (3.3 x 10^7 CFU kg^{-1} of *Bacillus* spp.); 3) commercial feed with probiotic *L. plantarum* + *Bacillus* spp. (10^7 CFU kg^{-1} of *L. plantarum* + 3.3 x 10^7 CFU kg^{-1} of *Bacillus* spp.); and 4) commercial feed without probiotics as control diet.

The probiotic *L. plantarum* was added to shrimp feed by mixing 100 mL of probiotic per kilogram in the diet, according to the methodology described by Vieira et al. (2008). The commercial probiotic was added to feed adding one gram of probiotic diluted in 100 mL of fish oil and mixed homogeneously every 3 kg of feed. The same amount of fish oil was added to the diet of the control group. In treatments with addition of commercial *Bacillus* spp. (diets 2 and 3) the product was also added weekly to culture water (0.5 g m^{-3}), following the supplier's instructions.

A commercial feed containing 35% of crude protein (Guabi® - Poti Guaçu, 1.6 mm) was provided to the shrimp four times a day (8:00 am, 11:00 am, 2:00 pm and 5:00 pm), according to a feeding table (Van Wyk and Scarpa, 1999), for 50 days. The amount of feed was calculated according to biometrics measurements performed weekly, using a sample size of shrimp per tank.

Water quality

During the experimental period, dissolved oxygen and water temperature of each tank were checked twice a day (08:00h - 16:00 h), using the multi-parameter YSI 556 MPS. Water samples were collected weekly to determine the levels of total ammonia nitrogen (TAN), nitrite ($N\text{-}NO_2$), nitrate ($N\text{-}NO_3$), total suspended solids (TSS) (APHA, 2005), pH (pH-metro Tecnal®), alkalinity (APHA, 2005), and salinity (Eco-Sense YSI EC3).

The control of ammonia was performed with the addition of sugar cane as an organic carbon source at a ratio of 20 g carbohydrate for each 1 mg of TAN, in accordance with Avnimelech (1999). To adjust alkalinity levels, calcium hydroxide ($Ca(OH)_2$) was added when the values were below 150 mg $CaCO_3$ L^{-1}.

The excess of suspended solids was removed with of a conical settling chamber, using cylindrical-conical-shaped sedimenters coupled in parallel to the culture tank to maintain levels between 400 and 600 mg L^{-1} (Schveitzer et al., 2013).

Microbiological analysis

At the end of the experiment, a pool of 10 shrimp intestines and 10 mL of water from each tank were sampled for microbiological analysis.

The intestines and water were homogenized and serially diluted (1/10) in sterile saline solution 3%. The samples were inoculated by using the spread plaque technique in the culture media: marine agar, TCBS agar (thiosulfate, citrate, bile and sucrose) and MRS agar for total heterotrophic bacteria, Vibrionaceae and lactic acid bacteria counting, respectively.

The samples inoculated in marine agar and TCBS were incubated at 30°C for 24 hours, and the samples on MRS agar were incubated at 35ºC for 48 hours. After this period, colonies were counted and reported as colony forming units (CFU).

Growth performance

At the end of the experiment, the final mean weight (g), total weight gain (g), survival (%), final biomass (kg), final productivity (kg m^{-3}) and feed conversion rate (FCR) of the animals were determined using the following equations:

Final mean weight (g) = total shrimp biomass / total number of shrimp;

Total weight gain (g) = average of final total weight – average of initial total weight;

Survival (%) = (final shrimp population - initial population) x 100;

Final biomass (kg) = average weight x final population;

Final productivity (kg m^{-3}) = total biomass / area;

FCR = feed intake (g)/ final biomass (g).

Statistical analysis

Data were analyzed using one-way ANOVA with repeated measures. The Tukey test was applied to verify the significant differences. Homoscedasticity and normality were confirmed using the Levene and Shapiro-Wilk tests, respectively. All statistical tests were evaluated with a significance level of $P < 0.05$, using the STATISTICA software, version 8.0.

RESULTS

Growth performance

The control group and treatment with *L. plantarum* resulted in higher final weight, weight gain, final biomass and final productivity compared to the other treatments (Table 1). The highest FCR and the lowest survival were obtained in the *L. plantarum* + *Bacillus* spp. treatment.

Water quality

The physicochemical variables of water remained relatively stable throughout the experiment (Table 2). The values of salinity (33.56 ± 0.17 g L^{-1}), pH (7.47 ± 0.04) and alkalinity (172 ± 2.94 mg $CaCO_3$ L^{-1}) did not present significant differences since all values were within the acceptable range for cultivation of marine shrimp (Diaz and Rosenberg, 1995; Van Wyk and Scarpa, 1999).

Table 1. Growth performance of *Litopenaeus vannamei* after 50 days of rearing in a biofloc system (mean ± standard deviation).

Performance	**Treatments**			
	Control	***L. plantarum***	***Bacillus* spp.**	***L. plantarum* + *Bacillus* spp.**
Final weight (g)	10.14±0.71[a]	9.78±0.48[a]	7.56±0.68[b]	6.06±0.81[b]
Total weight gain (g)	8.16±0.31[a]	7.80±0.37[a]	5.55±0.56[b]	4.04±0.24[b]
Survival (%)	86.96±4.63[a]	84.45±6.49[a]	83.69±4.65[a]	57.64±5.77[b]
Final biomass (kg)	23.75±0.36[a]	22.26±0.92[a]	19.08±0.81[b]	9.50±2.19[c]
Final productivity (kg m^{-3})	2.97±0.04[a]	2.78±0.11[a]	2.39±0.10[b]	1.19±0.27[c]
FCR	1.86±0.03[a]	1.96±0.15[a]	2.26±0.16[a]	8.49±4.61[b]

Different letters in rows indicate significant differences by the Tukey test at 5% probability.

Table 2. Water quality variables for *Litopenaeus vannamei* reared in a biofloc system with different treatments (mean ± standard deviation).

Variables	Treatments			
	Control	*L. plantarum*	***Bacillus*** **spp.**	***L. plantarum*** **+ *Bacillus*** **spp.**
pH	7.47±0,04	7.48±0.07	7.51±0.07	7.42±0.05
Salinity	33.62±0.43	33.78±0.05	33.41±0.04	33.43±0.01
Alkalinity (mg $CaCO_3$ L^{-1})	169 ±0,32	172 ±1.05	176±1.13	171±1.45
Ammonia N-NH_3 (mg L^{-1})	3.63±0.09	4.17±0.49	3.71±0.48	3.77±0.17
Nitrite N-NO_2 (mg L^{-1})	1.76±0.45^a	1.75±0.48^a	1.44±1.58^a	10.7±1.23^b
TSS (mg L-^{1})	762±236.70	574±4.45	573±104.47	773±27.93

Different letters in rows indicate significant differences by the Tukey test at 5% probability.

TSS did not show significant differences during the experiment; however, TSS did remain above the recommended levels for the species (Ray et al., 2010). Total ammonia (N-NH_3) showed no significant differences among treatments and presented an average value of 3.82 ± 0.24 mg L^{-1}, which is also above recommended levels for the species. The *L. plantarum* + *Bacillus* spp. treatment showed the highest nitrite (N-NO_2) values, differing statistically from the other groups. Temperature and dissolved oxygen were maintained at 28.52 ± 0.64ºC and 6.47 ± 0.35 mg L^{-1}, respectively, with no differences among treatments.

Microbiological count of water

The total heterotrophic bacteria counts were similar among all treatments (Figure 1A). Treatment with the probiotic *L. plantarum*

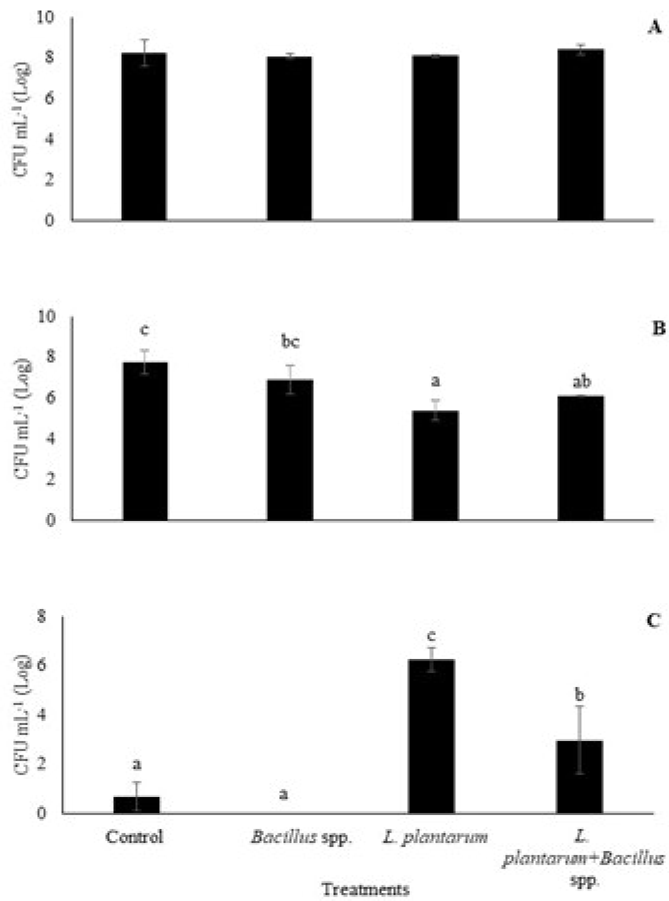

Figure 1. Counts of total heterotrophic bacteria (A), *Vibrio* spp. (B) and lactic acid bacteria (C) from culture water for *L. vannamei* reared in a biofloc system with different treatments after 50 days. Different letters in the bars indicate significant differences by the Tukey test at 5% probability.

resulted in the lowest counts of *Vibrio* spp. compared to the use of the commercial probiotic *Bacillus* spp. and the control group (Figure 1B). The use of *L. plantarum* alone did not differ from the use of the two probiotics combined (*L. plantarum* + *Bacillus* spp.), which resulted in similar counts.

Counts of LAB were higher in treatments using *L. plantarum* alone compared to the other treatments (Figure 1C). In contrast, no presence of LAB was detected in treatments that received the commercial probiotic with *Bacillus* spp.

Microbiological count of intestinal tract

The counts of total heterotrophic bacteria were similar among treatments, differing only between the *Bacillus* spp. treatment and the *L. plantarum* + *Bacillus* spp. treatment that resulted in higher counts by use of the combined probiotics (Figure 2A).

The probiotic *L. plantarum* used alone resulted in the highest counts of lactic acid bacteria and significantly reduced the presence of *Vibrio* spp. in shrimp intestine counts compared to the other treatments (Figure 2B). No presence of LAB was detected in the intestine counts with *Bacillus* spp. treatments (Figure 2C).

DISCUSSION

Probiotics are widely recognized in aquaculture production for increasing growth rate, improving digestion, and providing nutritional factors to the host. Lactic bacteria and *Bacillus* are the most used bacterial strains in aquaculture. Several studies report positive results with the use of these strains (endogenous or exogenous, mono, or multispecies) on growth, survival and/or feed conversion ratio in marine shrimp (Kumar et al., 2014; Sánchez-Ortiz et al., 2016; Franco et al., 2017; Hostins et al., 2017; Zheng et al., 2017; Nguyen et al., 2018; Kewcharoen and Srisapoome, 2019; Xie et al., 2019; Zuo et al., 2019; Wang et al., 2020; Won et al., 2020).

In the present study, the growth performance obtained with the use of *L. plantarum* was not affected with results similar to those obtained in the control group. On the other hand, the two treatments with addition of commercial probiotic containing *Bacillus* spp. presented a lower performance. According with some authors, foreign bacteria have a reduced capacity to remain viable because they are non-host-associated strains, thus decreasing their beneficial effect, or, in some cases, producing undesirable

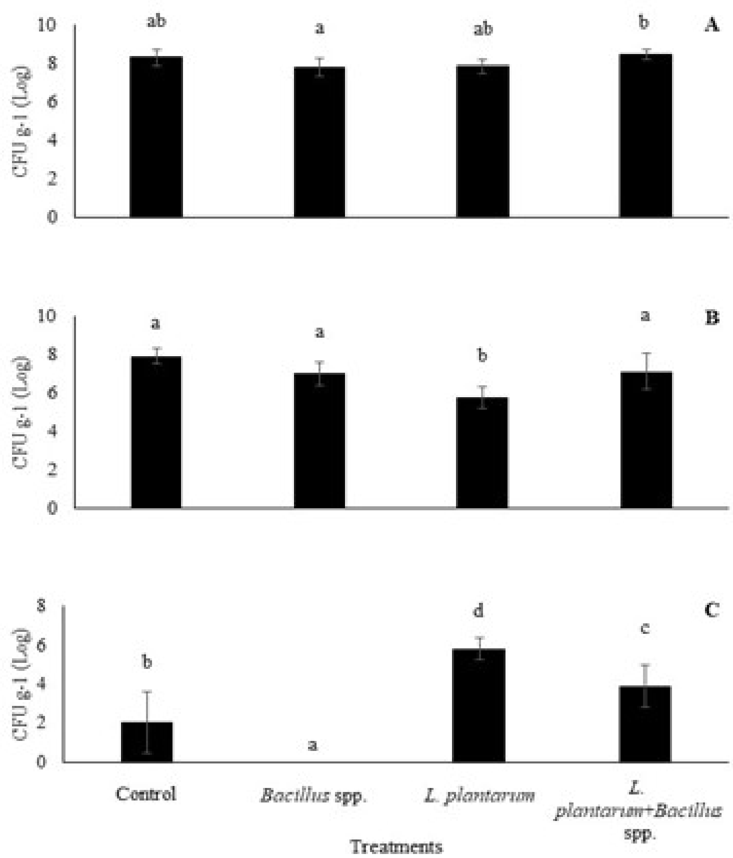

Figure 2. Intestinal counts of total heterotrophic bacteria (A), *Vibrio* spp. (B) and lactic acid bacteria (C) of *Litopenaeus vannamei* fed different diets for 50 days in a biofloc system. Different letters in the bars indicate significant differences by the Tukey test at 5% probability.

effects (Nayak, 2010; Lazado et al., 2015; Jamal et al., 2019) as the results obtained in the present study. As an example, a study carried out in a BFT system using commercial probiotic (*Bacillus* spp., Lactic acid, *Lactobacillus* spp., and *Saccharomyces* spp.) did not improve the growth performance of *L. vannamei*, and the specific growth rate was lower than had already been obtained in experimental hatcheries in the same system (Arias-Moscoso et al., 2018).

On the other hand, Franco et al. (2017) demonstrated that the use of an indigenous intestinal strain of *L. vannamei* (*B. liqueniformis*) resulted in better quality of shrimp post-larvae than the use of a commercial multispecies probiotic. They suggest that indigenous bacterium of the intestinal tract, has better adaptation, resulting in a better growth performance of the animals, when compared to the commercial probiotic. Although the present study did not obtain better results in the growth performance with the indigenous probiotic, its use did not negatively affect the shrimps.

To obtain a better effect of probiotics, several studies using combinations of lactic bacteria and *Bacillus* spp. reported improvements in shrimp performance, in some cases with even better results than those obtained with the strains used separately, indicating a synergistic effect (Kumar et al., 2014; Sánchez-Ortiz et al., 2016; Hostins et al., 2017; Xie et al., 2019; Wang et al., 2019; Wang et al., 2020). However, contrary to what was expected, the combined use of *L. plantarum* and *Bacillus* spp. in the present study showed a negative effect on feed conversion ratio and resulted in lower survival rates than probiotics used separately. The low productivity and final biomass obtained with *Bacillus* spp., as well as the high rate of feed conversion using lactic bacteria and commercial product combined, could be related to the lowest survival obtained.

Probiotic addition directly to the rearing water is also a strategy to obtain benefits in shrimp production through the modulation of water quality parameters. Probiotic bacteria, especially *Bacillus* spp., are widely used as bioremediators to decrease excess organic matter and toxic compounds, such as those that are nitrogenous (Soltani et al., 2019; Ringø, 2020), the main concern in biofloc systems after those that are oxygenous (Emerenciano et al., 2017; Soltani et al., 2019).

In the present study, the commercial product (*Bacillus* spp.) was incorporated into both feed and water, according to the supplier's instructions. Although ammonia reached levels above the ideal for shrimp rearing, survival was not affected in the control group or the groups reared on *L. plantarum* and *Bacillus* spp., reaching values similar to those of other studies carried out with BFT (Furtado et al., 2011; Krummenauer et al., 2014; Kewcharoen and Srisapoome, 2019). However, the high nitrite levels achieved with the *L. plantarum* + *Bacillus* spp. treatment can be explained by the high mortality that resulted. The biofloc water did not allow to see the dead animals and the supply of food continued to be high, consequently increasing the nitrite levels in the water (Emerenciano et al., 2017).

On the other hand, the TSS levels were higher than recommended for *L. vannamei* farming (500 mg L^{-1}), mainly in the control group and *L. plantarum* + *Bacillus* spp. treatment, but without affecting shrimp growth performance. In BFT systems, the addition of commercial bacteria consortia is done to improve the control of nitrogen levels and solid compounds, recycling organic matter, and stabilizing the heterotrophic bacteria community (Emerenciano et al., 2017). However, the commercial probiotic tested in the present study did not improve the water quality of the system, despite being composed of bacteria known for their bioremediative power. Likewise, the tanks that received lactic bacteria through feeding did not show changes in the water variables measured, which was expected because lactic bacteria are mainly used to obtain physiological and immunological responses in shrimp. For this reason, lactic acid bacteria were only added to feed.

In the present study, BHT counts in the culture water were similar between the control group and the treatments that received probiotic bacteria. However, addition of probiotic bacteria to the culture system does not necessarily increase the total bacterial concentrations. In fact, it can alter microbial diversity (Vargas-Albores et al., 2017; Huerta-Rábago et al., 2019). Although we did not carry out analyses to determine bacterial diversity, our results indicated differences in *Vibrio* spp. and lactic bacteria counting. The lowest concentrations of *Vibrio* spp. were obtained in treatments with *L. plantarum* and *L. plantarum* +*Bacillus* spp. This can be explained by the inhibitory action of lactic bacteria alone and not by commercial probiotic since the individual use of commercial *Bacillus* spp. did not decrease the concentrations of *Vibrio* spp. In addition, results indicated no synergistic effect between the two probiotics tested since the concentration of *Vibrio* spp. was similar between them.

The inhibition of *Vibrio* spp. by *L. plantarum* has already been demonstrated in *in vitro* and *in vivo* tests carried out using this same strain (Vieira et al., 2008, 2010; Ramírez et al., 2013). The commercial *Bacillus* spp. did not influence *Vibrio* spp. concentration in the water, perhaps by the lack of adaptation to the system. *Vibrio* spp. are nonnative bacteria, as previously suggested. However, results obtained in a previous study carried out by our research group in the same facilities showed that *Bacillus* spp. isolated from the BFT culture system could decrease the concentrations of *Vibrio* spp. in the water (Ferreira et al., 2015). This result suggests that isolated bacteria from the environment itself could have more appropriate and positive effect on our system. However, this does not mean that commercial probiotics with nonnative microorganisms do not have beneficial effects, as shown by several studies. As an example, a study carried out in a BFT system with *L. vannamei* obtained lower concentrations of *Vibrio* spp. in the water when two commercial probiotics that contained a mixture of different microorganisms were added (Arias-Moscoso et al., 2018). Likewise, Hostins et al. (2017) reported that the use of a commercial probiotic containing several species of *Bacillus* reduced the presence of *Vibrio* spp. in the culture water of *L. vannamei* in BFT when compared to treatments that did not receive probiotic. Therefore, it is important to make sure that commercial probiotic microorganisms can adapt to the system to be incorporated, as many are used indiscriminately in different environmental conditions (Uddin et al., 2015).

Despite similarities in the BHT intestinal counts in all treatments, the shrimp fed only *L. plantarum* showed lower concentrations of *Vibrio* spp. As previously suggested, *L. plantarum* is a bacterium isolated from the intestinal tract of *L. vannamei* and may present better adaptation by modifying the intestinal microbiota inhibiting different pathogens. This was verified in a study made by Zheng et al. (2017) where they found that the intestinal microbiota of shrimp is modified when supplemented with *L. plantarum*, increasing populations of bacteria known to inhibit different pathogens, and according with Kongnum and Hongpattarakere (2012) LAB population could be established as dominant flora through feeding. Some studies using different probiotic bacteria together have obtained good results by decreasing the concentration of *Vibrio* spp. in the intestinal tract of shrimp (Boonthai et al., 2011; Bernal et al., 2017). However, the use of *L. plantarum* alone proved to be more effective in reducing the concentrations of *Vibrio* spp. in the intestinal tract compared to its combined use with the commercial product. Probiotic bacteria like *L. plantarum* and *Bacillus*, besides competing for space and nutrients, are known to produce antimicrobial compounds (Abriouel et al., 2011; Ringø et al., 2020) that can result in antagonism between them. According to the results obtained, lactic bacteria concentrations decreased when *L. plantarum* was added in combination with the commercial product, in both water and intestinal tract, suggesting that the presence of *Bacillus* spp. can inhibit this bacterial group. This antagonism decreases *L. plantarum* concentrations and, consequently, its inhibitory capacity, explaining the higher intestinal concentrations of *Vibrio* spp. in the groups that received the two probiotics combined. This should serve as a warning against the indiscriminate use of probiotic mixtures of different commercial products since the use of many different strains can often be dispensable.

As expected, the presence of lactic bacteria in the culture water and shrimp intestine in the control group indicates that they are part of the local system and the intestinal microbiota of shrimp. The absence of this bacterial group in the treatment using only the commercial product suggests that this probiotic inhibits populations of lactic bacteria by altering the native bacterial community of shrimp. In addition to inhibiting endogenous bacteria that may play a relevant role in modulating the present community, the use of bacteria that are nonnative to both the host and the system may act as immunostimulants, resulting in unnecessary energy expenditure that would affect the animals' performance (Vargas-Albores et al., 2017).

Thus, we suggest that the use of host-associated bacteria would be more beneficial, helping to reduce environmental imbalance resulting from the introduction of nonnative microorganisms, and, at the same time, efficiently adapting to the culture system bringing better results (Ringø et al., 2020). It is also important that *in vitro* tests be carried out to find bacterial combinations that really have a synergistic effect and do not present antagonism in order to maximize the beneficial effects of using several probiotics together (Timmerman et al., 2004) to not generating unnecessary production expenses.

CONCLUSION

Use of the *L. plantarum* strain alone decreased the concentration of *Vibrio* spp., modifying the intestinal microbiota, but without affecting growth performance. In contrast, the use of *Bacillus* spp. combined with *L. plantarum* negatively influenced the survival and feed conversion ratio and significantly increased the nitrite levels of water. At the same time, though, it presented an antagonistic effect against lactic bacteria, decreasing its concentrations, both in intestine and water. Control treatment has the highest concentration of *Vibrio* spp. in water in turn without affecting shrimp performance. In summary, the combined use of the two probiotics did not have any positive effects on the cultivation of *L. vannamei* in our model BFT system.

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REFERENCES

Abriouel, H.; Franz, C.M.; Omar, N.B.; Gálvez, A. 2011. Diversity and applications of Bacillus bacteriocins. FEMS Microbiology Reviews, 35(1): 201-232. https://doi.org/**10.1111/j.1574-6976.2010.00244.x**.

APHA – American Public Health Association, American Water Works Association and Water Pollution Control Association. 2005. Standard methods for the examination of water and wastewater. 21st ed. Washington: American Public Health Association.

Arias-Moscoso, J.L.; Espinoza-Barrón, L.G.; Miranda-Baeza, A.; Rivas-Vega, M.E.; Nieves-Soto, M. 2018. Effect of commercial probiotics addition in a biofloc shrimp farm during the nursery phase in zero water exchange. Aquaculture Reports, 11: 47-52. https://doi.org/**10.1016/j.aqrep.2018.06.001**.

Avnimelech, Y. 1999. Carbon/nitrogen ratio as control element in aquaculture systems. Aquaculture, 176(3-4): 227-235. https://doi.org/**10.1016/S0044-8486(99)00085-X**.

Bernal, M.G.; Marrero, R.M.; Campa-Córdova, Á.I.; Mazón-Suástegui, J.M. 2017. Probiotic effect of *Streptomyces* strains alone or in combination with *Bacillus* and *Lactobacillus* in juveniles of the white shrimp *Litopenaeus vannamei*. Aquaculture International, 25(2): 927-939. https://doi.org/**10.1007/s10499-016-0085-y**.

Boonthai, T.; Vuthiphandchai, V.; Nimrat, S. 2011. Probiotic bacteria effects on growth and bacterial composition of black tiger shrimp (*Penaeus monodon*). Aquaculture Nutrition, 17(6): 634-644. https://doi.org/**10.1111/j.1365-2095.2011.00865.x**.

Diaz, R.J.; Rosenberg, R. 1995. Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanography and Marine Biology, 33: 245-303.

Emerenciano, M.G.C.; Martínez-Córdova, L.R.; Martínez-Porchas, M.; Miranda-Baeza, A. 2017. Biofloc Technology (BFT): a tool for water quality management in aquaculture. In: Tutu, H. Water quality. London: IntechOpen. https://doi.org/**10.5772/66416**.

FAO – Food and Agriculture Organization of the United Nations. 2020. The state of world fisheries and aquaculture 2020. Sustainability in action. Rome: FAO. 224p. https://doi.org/**10.4060/ca9229en**.

Ferreira, G.S.; Bolívar, N.C.; Pereira, S.A.; Guertler, C.; Vieira, F.N.; Seiffert, W.Q. 2015. Microbial biofloc as source of probiotic bacteria for the culture of *Litopenaeus vannamei*. Aquaculture, 448: 273-279. https://doi.org/**10.1016/j.aquaculture.2015.06.006**.

Franco, R.; Martín, L.; Arenal, A.; Santiesteban, D.; Sotolongo, J.; Cabrera, H.; Castillo, N.M. 2017. Evaluation of two probiotics used during farm production of white shrimp *Litopenaeus vannamei* (Crustacea: Decapoda). Aquaculture Research, 48(4): 1936-1950. https://doi.org/**10.1111/are.13031**.

Furtado, P.S.; Poersch, L.H.; Wasielesky, W. 2011. Effect of calcium hydroxide, carbonate and sodium bicarbonate on water quality and zootechnical performance of shrimp *Litopenaeus vannamei* reared in bio-flocs technology (BFT) systems. Aquaculture, 321(1-2): 130-135. https://doi.org/**10.1016/j.aquaculture.2011.08.034**.

Gopal, S.; Otta, S.K.; Kumar, S.; Karunasagar, I.; Nishibuchi, M.; Karunasagar, I. 2005. The occurrence of *Vibrio* species in tropical shrimp culture environments; implications for food safety. International Journal of Food Microbiology, 102(2): 151-159. https://doi.org/**10.1016/j.ijfoodmicro.2004.12.011**.

Gullian, M.; Thompson, F.; Rodriguez, J. 2004. Selection of probiotic bacteria and study of their immunostimulatory effect in *Penaeus vannamei*. Aquaculture, 233(1-4): 1-14. https://doi.org/**10.1016/j.aquaculture.2003.09.013**.

Hostins, B.; Lara, G.; Decamp, O.; Cesar, D.E.; Wasielesky, W. 2017. Efficacy and variations in bacterial density in the gut of *Litopenaeus vannamei* reared in a BFT system and in clear water supplemented with a commercial probiotic mixture. Aquaculture, 480: 58-64. https://doi.org/**10.1016/j.aquaculture.2017.07.036**.

Huerta-Rábago, J.A.; Martínez-Porchas, M.; Miranda-Baeza, A.; Nieves-Soto, M.; Rivas-Vega, M.E.; Martínez-Córdova, L.R. 2019. Addition of commercial probiotic in a biofloc shrimp farm of *Litopenaeus vannamei* during the nursery phase: effect on bacterial diversity using massive sequencing 16S rRNA. Aquaculture, 502: 391-399. https://doi.org/**10.1016/j.aquaculture.2018.12.055**.

Jamal, M.T.; Abdulrahman, I.A.; Al Harbi, M.; Chithambaran, S. 2019. Probiotics as alternative control measures in shrimp aquaculture: A review. Journal of Applied Biology and Biotechnology, 7(3): 69-77. https://doi.org/**10.7324/JABB.2019.70313**.

Kewcharoen, W.; Srisapoome, P. 2019. Probiotic effects of *Bacillus* spp. from Pacific white shrimp (*Litopenaeus vannamei*) on water quality and shrimp growth, immune responses, and resistance to *Vibrio parahaemolyticus* (AHPND strains). Fish & Shellfish Immunology, 94: 175-189. https://doi.org/**10.1016/j.fsi.2019.09.013**.

Knipe, H.; Temperton, B.; Lange, A.; Bass, D.; Tyler, C.R. 2021. Probiotics and competitive exclusion of pathogens in shrimp aquaculture. Reviews in Aquaculture, 13(1): 324-352. https://doi.org/**10.1111/raq.12477**.

Kongnum, K.; Hongpattarakere, T. 2012. Effect of *Lactobacillus plantarum* isolated from digestive tract of wild shrimp on growth and survival of white shrimp (*Litopenaeus vannamei*) challenged with *Vibrio harveyi*. Fish & Shellfish Immunology, 32(1): 170-177. https://doi.org/**10.1016/j.fsi.2011.11.008**.

Krummenauer, D.; Samocha, T.; Poersch, L.; Lara, G.; Wasielesky, W. 2014. The reuse of water on the culture of pacific white shrimp, *Litopenaeus vannamei*, in BFT System. Journal of the World Aquaculture Society, 45(1): 3-14. https://doi.org/**10.1111/jwas.12093**.

Kumar, A.; Babu, P.P.S.; Roy, S.D.; Razvi, S.S.; Charan, R. 2014. Synergistic effects of two probiotic bacteria on growth, biochemical, and immunological responses of *Litopenaeus vannamei* (Boone, 1931). Israeli Journal of Aquaculture - Bamidgeh, 66: 1-8.

Lazado, C.C.; Caipang, C.M.A.; Estante, E.G. 2015. Prospects of host-associated microorganisms in fish and penaeids as probiotics with immunomodulatory functions. Fish & Shellfish Immunology, 45(1): 2-12. https://doi.org/**10.1016/j.fsi.2015.02.023**.

Merrifield, D.L.; Dimitroglou, A.; Foey, A.; Davies, S.J.; Baker, R.R.; Bøgwald, J.; Castex, M.; Ringø, E. 2010. The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture, 302(1-2): 1-18. https://doi.org/**10.1016/j.aquaculture.2010.02.007**.

Nayak, S.K. 2010. Probiotics and immunity: a fish perspective. Fish & Shellfish Immunology, 29(1): 2-14. https://doi.org/**10.1016/j.fsi.2010.02.017**.

Nguyen, T.T.G.; Nguyen, T.C.; Leelakriangsak, M.; Pham, T.T.; Pham, Q.H.; Lueangthuwapranit, C. 2018. Promotion of *Lactobacillus plantarum* on growth and resistance against acute hepatopancreatic necrosis disease pathogens in white-leg shrimp (*Litopenaeus vannamei*). Wetchasan Sattawaphaet, 48(1): 19-28.

Ramírez, N.B.; Seiffert, W.Q.; Vieira, F.D.N.; Mourino, J.L.P.; Jesus, G.F.A.; Ferreira, G.S.; Andreatta, E.R. 2013. Dieta suplementada com prebiótico, probiótico e simbiótico no cultivo de camarões marinhos. Pesquisa Agropecuária Brasileira, 48(8): 913-919. https://doi.org/**10.1590/S0100-204X2013000800015**.

Ray, A.J.; Seaborn, G.; Leffler, J.W.; Wilde, S.B.; Lawson, A.; Browdy, C.L. 2010. Characterization of microbial communities in minimal-exchange, intensive aquaculture systems and the effects of suspended solids management. Aquaculture, 310(1-2): 130-138. https://doi.org/**10.1016/j.aquaculture.2010.10.019**.

Ringø, E. 2020. Probiotics in shellfish aquaculture. Aquaculture and Fisheries, 5(1): 1-27. https://doi.org/**10.1016/j.aaf.2019.12.001**.

Ringø, E.; Van Doan, H.; Lee, S.H.; Soltani, M.; Hoseinifar, S.H.; Harikrishnan, R.; Song, S.K. 2020. Probiotics, lactic acid bacteria and bacilli: interesting supplementation for aquaculture. Journal of Applied Microbiology, 129(1): 116-136. https://doi.org/**10.1111/jam.14628**.

Sánchez-Ortiz, A.C.; Angulo, C.; Luna-González, A.; Álvarez-Ruiz, P.; Mazón-Suástegui, J.M.; Campa-Córdova, Á.I. 2016. Effect of mixed-*Bacillus* spp. isolated from pustulose ark *Anadara tuberculosa* on growth, survival, viral prevalence and immune-related gene expression in shrimp *Litopenaeus vannamei*. Fish & Shellfish Immunology, 59: 95-102. https://doi.org/**10.1016/j.fsi.2016.10.022**.

Schveitzer, R.; Arantes, R.; Costódio, P.F.S.; Santo, C.M.E.; Arana, L.V.; Seiffert, W.Q.; Andreatta, E.R. 2013. Effect of different biofloc levels on microbial activity, water quality and performance of *Litopenaeus vannamei* in a tank system operated with no water exchange. Aquacultural Engineering, 56: 59-70. https://doi.org/**10.1016/j.aquaeng.2013.04.006**.

Skjermo, J.; Vadstein, O. 1999. Techniques for microbial control in the intensiverearing of marine larvae. Aquaculture, 177(1-4): 333-343. https://doi.org/**10.1016/S0044-8486(99)00096-4**.

Soltani, M.; Ghosh, K.; Hoseinifar, S.H.; Kumar, V.; Lymbery, A.J.; Roy, S.; Ringø, E. 2019. Genus *Bacillus*, promising probiotics in aquaculture: Aquatic animal origin, bio-active components, bioremediation and efficacy in fish and shellfish. Reviews in Fisheries Science & Aquaculture, 27(3): 331-379. https://doi.org/**10.1080/23308249.2019.1597010**.

Timmerman, H.M.; Koning, C.J.M.; Mulder, L.; Rombouts, F.M.; Beynen, A.C. 2004. Monostrain, multistrain and multispecies probiotics—a comparison of functionality and efficacy. International Journal of Food Microbiology, 96(3): 219-233. https://doi.org/**10.1016/j.ijfoodmicro.2004.05.012**.

Uddin, G.M.; Larsen, M.H.; Christensen, H.; Aarestrup, F.M.; Phu, T.M.; Dalsgaard, A. 2015. Identification and antimicrobial resistance of bacteria isolated from probiotic products used in shrimp culture. PLoS One, 10(7): e0132338. https://doi.org/**10.1371/journal.pone.0132338**.

Van Wyk, P.; Scarpa, J. 1999. Water quality requirements and management. In: Van Wyk, P.; Davis-Hodgkins, M.; Laramore, R.; Main, K.L.; Mountain, J.; Scarpa, J. Farming marine shrimp in recirculating freshwater systems. Tallahassee: Florida Department of Agriculture and Consumer Services. pp.141-162.

Vargas-Albores, F.; Porchas-Cornejo, M.A.; Martínez-Porchas, M.; Villalpando-Canchola, E.; Gollas-Galván, T.; Martínez-Córdova, L.R. 2017. Bacterial biota of shrimp intestine is significantly modified by the use of a probiotic mixture: a high throughput sequencing approach. Helgoland Marine Research, 71(1): 1-10. https://doi.org/**10.1186/s10152-017-0485-z**.

Verschuere, L.; Rombaut, G.; Sorgeloos, P.; Verstraete, W. 2020. Probiotics bacteria as biological control agents in aquaculture. Microbiology and Molecular Biology Reviews, 64(4): 655-671. https://doi.org/**10.1128/MMBR.64.4.655-671.2000**.

Vieira, F.D.; Buglione, C.C.; Mourino, J.L.P.; Jatobá, A.; Ramirez, C.; Martins, M.L.; Barracco, M.A.A.M.; Vinatea, L.A. 2008. Time-related action of *Lactobacillus plantarum* in the bacterial microbiota of shrimp digestive tract and its action as immunostimulant. Pesquisa Agropecuária Brasileira, 43(6): 763-769. https://doi.org/**10.1590/S0100-204X2008000600013**.

Vieira, F.N.; Buglione, C.C.; Mouriño, J.P.L.; Jatobá, A.; Martins, M.L.; Schleder, D.D.; Andreatta, E.R.; Barraco, M.A.; Vinatea, L.A. 2010. Effect of probiotic supplemented diet on marine shrimp survival after challenge with *Vibrio harveyi*. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 62(3): 631-638. https://doi.org/**10.1590/S0102-09352010000300019**.

Wang, Y.; Al Farraj, D.A.; Vijayaraghavan, P.; Hatamleh, A.A.; Biji, G.D.; Rady, A.M. 2020. Host associated mixed probiotic bacteria induced digestive enzymes in the gut of tiger shrimp *Penaeus monodon*. Saudi Journal of Biological Sciences, 27(9): 2479-2484. https://doi.org/**10.1016/j.sjbs.2020.07.010**.

Wang, Y.C.; Hu, S.Y.; Chiu, C.S.; Liu, C.H. 2019. Multiple-strain probiotics appear to be more effective in improving the growth performance and health status of white shrimp, *Litopenaeus vannamei*, than single probiotic strains. Fish & Shellfish Immunology, 84: 1050-1058. https://doi.org/**10.1016/j.fsi.2018.11.017**.

Wasielesky, W.; Atwood, H.; Stokes, A.; Browdy, C.L. 2006. Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. Aquaculture, 258(1-4): 396-403. https://doi.org/**10.1016/j.aquaculture.2006.04.030**.

Won, S.; Hamidoghli, A.; Choi, W.; Bae, J.; Jang, W.J.; Lee, S.; Bai, S.C. 2020. Evaluation of potential probiotics *Bacillus subtilis* WB60, *Pediococcus pentosaceus*, and *Lactococcus lactis* on growth performance, immune response, gut histology and immune-related genes in whiteleg shrimp, *Litopenaeus vannamei*. Microorganisms, 8(2): 281. https://doi.org/**10.3390/microorganisms8020281**.

Xie, J.J.; Liu, Q.Q.; Liao, S.; Fang, H.H.; Yin, P.; Xie, S.W.; Tian, L.X.; Liu, Y.J.; Niu, J. 2019. Effects of dietary mixed probiotics on growth, non-specific immunity, intestinal morphology and microbiota of juvenile pacific white shrimp, *Litopenaeus vannamei*. Fish & Shellfish Immunology, 90: 456-465. https://doi.org/**10.1016/j.fsi.2019.04.301**.

Zheng, X.; Duan, Y.; Dong, H.; Zhang, J. 2017. Effects of dietary *Lactobacillus plantarum* in different treatments on growth performance and immune gene expression of white shrimp *Litopenaeus vannamei* under normal condition and stress of acute low salinity. Fish & Shellfish Immunology, 62: 195-201. https://doi.org/**10.1016/j.fsi.2017.01.015**.

Zuo, Z.H.; Shang, B.J.; Shao, Y.C.; Li, W.Y.; Sun, J.S. 2019. Screening of intestinal probiotics and the effects of feeding probiotics on the growth, immune, digestive enzyme activity and intestinal flora of *Litopenaeus vannamei*. Fish & Shellfish Immunology, 86: 160-168. https://doi.org/**10.1016/j.fsi.2018.11.003**.