Latin American Journal of Aquatic Research, 52(4): 618-630, 2024
DOI: 10.3856/vol52-issue4-fulltext-3161

Research Article

Use of probiotic consortium technology during the grow-out of *Penaeus vannamei* juveniles (Boone, 1931) in a biofloc system

Amanda Favetta[1], Fabrício Martins Dutra[1], Caio Henrique do Nascimento Ferreira[1] Luisa Helena Cazarolli[2] & Eduardo Luis Cupertino Ballester[1]
[1]Laboratório de Carcinicultura, Programa de Pós-graduação em Aquicultura e Desenvolvimento Sustentável
Núcleo de Pesquisa e Desenvolvimento em Aquicultura Sustentável
Universidade Federal do Paraná, Maripá, Paraná, Brasil
[2]Laboratório de Bioquímica e Genética, Universidade Federal da Fronteira Sul
Laranjeiras do Sul, Laranjeiras do Sul, Paraná, Brasil
Corresponding author: Eduardo Luis Cupertino Ballester (elcballester@yahoo.com.br)

ABSTRACT. The objective was to evaluate the effect of probiotic consortium technology (PCT) on the performance of *Penaeus vannamei* juveniles in the biofloc system in a short time. The experiment had four treatments with four replicates. In each experimental unit, 35 animals (3.15 ± 0.53 g and 7.80 ± 0.54 cm) were stocked in a biofloc system for 35 days. The treatments were: control - control without probiotic; PCT1 - probiotic in the diet (3 mL kg^{-1}) and water (0.5 ppm - three times a week); PCT2 - probiotic in the diet (3 mL kg^{-1}) and water (1 ppm - daily), and PCT3 - probiotic in the water (1 ppm - daily). For water quality and *Vibrio*, no significant differences were observed among treatments ($P > 0.05$). Regarding the zootechnical performance, the treatments with the probiotic promoted higher final biomass, biomass gain, and a more efficient feed conversion rate ($P < 0.05$). The performance improvement promoted by the probiotic may be related to increased concentrations of amylases, chymotrypsin, and lipases in the hepatopancreas ($P < 0.05$). It was concluded that PCT2 promoted the best zootechnical performance of *P. vannamei* during the grow-out phase in the biofloc system.

Keywords: *Penaeus vannamei*; intensive system; BFT; enzyme activity; zootechnical performance; shrimp farming; aquaculture

INTRODUCTION

Biofloc technology (BFT) is an alternative for shrimp production in regions far from the sea, using artificially salinized water. The main benefit of this technology is minimum or zero water exchange (Emerenciano et al. 2017). It is a system that stimulates the formation of aggregates or biofloc, containing mainly heterotrophic and chemoautotrophic bacteria, microalgae, and other organisms. They convert excess nutrients in water into biomass, which also serves as food for organisms in the production system (Emerenciano et al. 2017, Almeida et al. 2021). Microorganisms formed in BFT can also help fight pathogens by competitive exclusion or by stimulating the immune system of crustaceans in rearing systems (Aalimahmoudi et al. 2017, Emerenciano et al. 2017).

The use of probiotics has been evaluated for BFT (Emerenciano et al. 2017, Arshad et al. 2018, Jiménez-Ordaz et al. 2021). Fuller (1989) defines probiotics as microbial food supplements that benefit the host. These supplements are commonly used in the diet or water to

Associate Editor: María Morales

stimulate biofloc formation, help stabilize water quality, and reinforce the microbial community, including resistance to *Vibrio* spp. (Ferreira et al. 2017). It is also possible to identify benefits for the morphology of the digestive tract, composition of the intestinal microbiota (Fan & Li 2019, Munaeni et al. 2020), immune response (Roomiani et al. 2018, Kewcharoen & Srisapoome 2019, Llario et al. 2019), digestive enzymatic activity and zootechnical performance of *Penaeus vannamei* (Amoah et al. 2019). Pacific white shrimp, *P. vannamei*, is the most produced crustacean in the world, representing 51.7% of production (5.8 million tons) in 2020 (FAO 2022). In the last two decades, increased demand for food, growing water scarcity, and environmental concerns have intensified shrimp production (Kumar et al. 2020, Xu et al. 2021).

The enzymatic activity (enzyme synthesis, secretion, and regulation) defines the digestive capacity (Carrillo-Farnés et al. 2007) and better nutrient absorption efficiency, which is reflected in better feed conversion and weight gain (Peixoto et al. 2018, Rocha et al. 2019). Microbial enzymes such as amylases, proteases, and lipases break down larger and more complex molecules, e.g. carbohydrates, proteins, and lipids, respectively (Tuan et al. 2013). Probiotics as a food supplement can improve enzyme activity and influence the composition of the intestinal microbial community (Adel et al. 2017).

Probiotic consortium technology (PCT) is an innovation based on the principle of active projected microbiomes, meaning that bacteria and yeast operate in a symbiotic environment in the right proportions and media to support and protect the production environment (George et al. 2016, Moreira et al. 2019, Rosado et al. 2023). Through the interaction of this system, internal and external, primary and secondary post-biotics are formed, which have direct effects on the environment, such as mineralization of organic matter, availability of nutrients, antimicrobial action (elements against pathogens), among others (Emerenciano et al. 2013, Arshad et al. 2018, Castellone 2022, Fathima et al. 2022).

The objective of this work was to evaluate the effect of PCT on water quality, control of *Vibrio* spp., zootechnical performance, and activity of digestive enzymes of *P. vannamei* juveniles during the growth phase in a biofloc system in a short time.

MATERIALS AND METHODS

The experiment was carried out at the Shrimp Culture Laboratory of the Center for Research and Development in Sustainable Aquaculture, Federal University of Paraná, Maripá - PR, for 35 days. The PCT evaluated was probiotic Bio O_2 Camarão®, composed of a mix of microorganisms based on lactic acid bacteria, yeasts, *Bacillus subtilis*, *Bifidobacterium animalis*, *Lactobacillus casei rhamnosus*, *Saccharomyces cerevisiae*, and 13 other species of bacteria used in the propagation process of PCT which, even in smaller amounts, are fundamental for the stabilization of the system, through the production of acids, sugars and metabolites.

Experimental design

The experimental design was completely randomized, with four treatments and four replicates, totaling 16 experimental units (EU). Thirty-five shrimp were stocked per EU (160 shrimp m^{-3} stocking density) with an average weight of 3.15 ± 0.53 g and average total length of 7.80 ± 0.54 cm, from the SpeedLine® strain of the company Aquatec®, Canguaretama, Rio Grande do Norte, Brazil. The production medium (bioflocs) and animals were obtained from a local producer. The biofloc (5,000 L) was transferred to the experiment site using mechanical aeration in a fish transport box. The biofloc, without the addition of probiotics, had gone through a denitrification process to reuse the water and was under development for the start of a commercial grow-out cycle for *P. vannamei*. The treatments were defined by evaluating the efficiency and cost of the product's application (Table 1).

Shrimp were stocked in circular polyethylene tanks with a volume of 220 L, arranged in a greenhouse in a static system with forced aeration. During the week before the beginning of the experiment, 10 g of carbon source (brown sugar) was added daily to each experimental unit to stimulate and maintain the formation of bioflocs. The units were covered with a shade cloth to prevent animals from escaping.

Feeding

In the first week, commercial diet Guabitech Inicial J® (40% crude protein; 2 mm) and Guabitech Active® (36% crude protein; 2 mm) were offered in a 3:7 ratio, respectively. Afterwards, only Guabitech Active® was offered. For the preparation of the rations, 3 mL kg^{-1} (3 mL of probiotic diluted in 100 mL of distilled water sprinkled on 1 kg of ration) was homogenized weekly and dried in an oven (approximately 35ºC) for 3 h, mixing the ration every 30 min for uniform drying. The rations without the probiotic went through the same process when the same volume of distilled water, without probiotics, was sprayed.

Table 1. Description of treatments regarding dosage and frequency of administration in water and inclusion in feed. PCT: probiotic consortium technology.

Treatment	Probiotic in water		Probiotic in feed
	Dosage	Frequency	
Control	No probiotic	-	No probiotic
PCT1	0.5 ppm	Three applications per week	3 mL kg^{-1} feed
PCT2	1 ppm	Seven applications per week	3 mL kg^{-1} feed
PCT3	1 ppm	Seven applications per week	No probiotic

Each experimental unit received 7.5 g of feed per day, divided into five feedings. The feed rate was calculated by projecting a growth of 1 g per week and a feed conversion of 1.5:1, providing the same amount throughout the entire duration of the experiment (Lara et al. 2017).

Water quality variables

Temperature (morning and afternoon) and dissolved oxygen (morning) were monitored daily with an oximeter (Hanna® - HI9146). The pH values were measured every four days with a pH Meter (Luca-210®) and salinity (manual refractometer - Atago®). Total hardness (titrimetric methods) and total alkalinity (titrimetric methods) were checked weekly (Baird et al. 2012).

The volume of sedimentable suspended solids (SSS) was quantified according to Avnimelech (2009) every five days. The methodology described in Baird et al. (2012) was applied on the 1st, 15th, and 31st days of the experiment to determine the total suspended solids (TSS), total ammonia (phenate methods), and nitrite (Griess reaction).

Vibrio analysis

Presumptive *Vibrio* colony-forming units (CFU) were analyzed in water and hepatopancreas samples. Plating and CFU counting were performed at the Experimental Nucleus of Applied Mycology (NEMA, by its Portuguese acronym), Federal University of Paraná, Palotina - PR.

Water samples from all experimental units were collected and immediately sent for plating on the experiment's 1st, 15th, and 31st day. Two dilutions of each sample were prepared, 1×10^{-1} and 1×10^{-2} (sample/0.85% saline solution), obtained by serial dilution. About 100 µL of each dilution was added to Petri dishes containing thiosulfate agar, citrate, bile, and sucrose culture medium. The plates were incubated in an oven at 37ºC (18 h), and after the incubation period, the CFUs were counted [adapted methodology (Vieira et al. 2010, Soto-Rodriguez et al. 2015)].

At the end of the experiment, the hepatopancreas of 10 animals from each EU were randomly collected. Hepatopancreas were stored in 1.5 mL microtubes and immediately sent to NEMA. The organs were weighed and homogenized in a vortex mixer with the addition of 0.85% saline solution (the amount of solution corresponding to the weight of each organ to obtain a dilution of 1×10^{-1} sample/saline solution). The plating, incubation, and CFU counting procedure was used for the water samples (Shanmugasundaram et al. 2015, Soto-Rodriguez et al. 2015).

Zootechnical performance

At the end of the experiment, all animals were counted, weighed (digital analytical scale Marte-AY220®, precision 0.0001 g), and measured (total length - ZAZ® caliper) to determine final average weight (FAW), final average length (FAL), survival (S), final biomass (FB), feed conversion rate (FCR) (Niu et al. 2014, Antunes et al. 2018), weight gain (WG), biomass gain (BG) (Niu et al. 2014), relative weight growth rate (RWGR), relative length growth rate (RLGR) (Steffens 1989), specific weight growth rate (SWGR) and specific length growth rate (SLGR) (Bautista-Teruel et al. 2003).

Evaluation of enzymatic activity

At the end of the experiment, the hepatopancreas of 10 animals per EU were randomly collected to evaluate the digestive enzyme activity. The hepatopancreas were placed separately in 1.5 mL microtubes and stored in liquid nitrogen. The analyses were conducted at the Biochemistry and Genetics Laboratory at the Federal University of Fronteira Sul, Laranjeiras do Sul, Paraná, Brazil.

For the analysis, hepatopancreas were homogenized in 8% saline solution with the aid of an electric homogenizer (IKA® T10 basic) and then centrifuged in a refrigerated centrifuge (Sigma, 3-16 KL) at 4°C for 10 min at 12,000 rpm. The supernatant obtained after centrifugation was removed and used for determi-

nations. The levels of protein (Bradford 1976), amylase and lipase (Seixas-Filho 2003), trypsin and chymotrypsin (Hummel 1959), and triacylglycerols (commercial kit, following the manufacturer's recommendations, Gold Analise®) were determined.

Statistical analysis

All variables underwent verification of normality and homogeneity assumptions, adopting a value of $\alpha = 5\%$ for all statistical tests. Values of temperature, dissolved oxygen, pH, salinity, total hardness, total alkalinity, enzymatic activity analysis, zootechnical performance, and CFU of *Vibrio* in hepatopancreas were subjected to one-way analysis of variance (ANOVA). Values of TSS, SSS, total ammonia, nitrite, and CFU of *Vibrio* in water were submitted to two-way ANOVA. The SSS values were submitted to nonpa-rametric Kruskal-Wallis analysis because they did not meet the assumptions of normality and homogeneity.

To meet the assumptions, the *Vibrio* CFU data in water and lipase activity underwent logarithmic transformation (log x). When differences were detected, the data were submitted to Tukey's mean comparison test.

RESULTS

Water quality

During the experimental period, no significant differences were observed among the treatments for the water quality variables monitored ($P > 0.05$). Results are summarized in Table 2.

The TSS concentration differed when considering each treatment over time ($P < 0.05$). It is possible to observe that between the samplings, there was an increase in concentrations in control and PCT2 (Fig. 1), and the last week of production was the period with the highest concentration of TSS (control: 576.7 mg L^{-1}, and PCT2: 450.0 mg L^{-1}). No differences were identified for PCT1 and PCT3 throughout the experimental period. Within the same sampling date, no differences were observed among treatments (Fig. 1; $P > 0.05$).

Figure 2 presents the SSS values. Comparison between treatments within each sampling did not show significant differences ($P > 0.05$). Considering each treatment over time, differences were observed for the control treatment between the first (average 0.3 mL L^{-1}) and the other sampling days (averages 0.725, 1.225, 2.55, 1.4, 1.875, and 13.225 mL L^{-1}, respectively; $P < 0.05$).

Total ammonia values during the experiment showed significant differences considering each treatment over time ($P < 0.05$). Treatments control, PCT1, and PCT2 showed a decrease, followed by an increase in the concentration of total ammonia during the experimental period, with the highest concentration of total ammonia in the last week (control - 0.0894 mg L^{-1}; PCT1 - 0.2108 mg L^{-1}, and PCT2 - 0.1931 mg L^{-1}). In the PCT3 treatment, the total ammonia concentrations did not show differences over time ($P > 0.05$). No differences were observed when comparing treatments on the same sampling date ($P > 0.05$; Fig. 3).

For nitrite concentration, when comparing each treatment during the same collection, no differences were identified (Fig. 4). The concentrations of nitrite in PCT1, PCT2, and PCT3 did not show a significant difference considering each treatment over time $P > 0.05$). At T1, a significant increase in nitrite concentration (0.0903 mg L^{-1}) was observed in the middle of the experiment relative to the beginning. However, in the last week, this compound's concentration was reduced ($P < 0.05$).

Vibrio monitoring

Regarding *Vibrio* in the water samples, there was no significant difference in CFU concentrations between treatments within each sampling date ($P > 0.05$). Considering each treatment throughout the experimental period, a difference was observed for PCT2 and PCT3 ($P < 0.05$). Both had the highest number of colonies on the second sampling date (Fig. 5).

The values to CFU of *Vibrio* spp. in *P. vannamei* hepatopancreas were 3.16 ± 2.66, 3.45 ± 3.06, 2.72 ± 1.45, and 3.53 ± 2.89 (1×10^4 CFU g^{-1}) to the control, PCT1, PCT2 and PCT3 treatments, respectively. There were no statistical differences among treatments ($P > 0.05$).

Zootechnical performance

The zootechnical variables such as FAW, FAL, S, WG, RWGR, RLGR, SWGR, and SLGR showed no difference among treatments ($P > 0.05$; Table 3).

Differences were observed among treatments for FB, BG, and feed conversion ($P < 0.05$). For FB, PCT2 presented the highest values, while for BG, the highest values were observed in PCT2 and PCT3. The lowest feed conversion values were observed at PCT2 and PCT3 (Table 3).

Table 2. Mean values ± standard deviation of water quality variables during the production of *P. vannamei* in a biofloc system with probiotic consortium technology (PCT) inclusion. *Recommended water quality values for *P. vannamei* (Van-Wyk et al. 1999, Rajkumar et al. 2016, Kumar et al. 2018). No statistical differences were detected among treatments ($P > 0.05$).

Variables	Control	PCT1	PCT2	PCT3	Recommended values*
Temperature (ºC)	27.3 ± 0.5	27.0 ± 0.4	27.0 ± 0.5	27.1 ± 0.4	28.0-32.0
Dissolved oxygen (mg L^{-1})	4.96 ± 0.07	4.94 ± 0.01	4.94 ± 0.08	5.01 ± 0.05	5.00-9.00
pH	9.04 ± 0.04	9.05 ± 0.01	9.03 ± 0.05	9.03 ± 0.03	7.00-8.30
Salinity	26.57 ± 0.41	26.64 ± 0.45	26.23 ± 0.33	26.26 ± 0.59	0.50-35.00
Total hardness (mg L^{-1} $CaCO_3$)	814.33 ± 136.60	953.33 ± 111.47	925.00 ± 133.70	716.67 ± 377.10	>100.00
Total alkalinity (mg L^{-1} $CaCO_3$)	132.88 ± 50.53	134.41 ± 47.62	138.29 ± 41.39	134.80 ± 42.26	>100.00

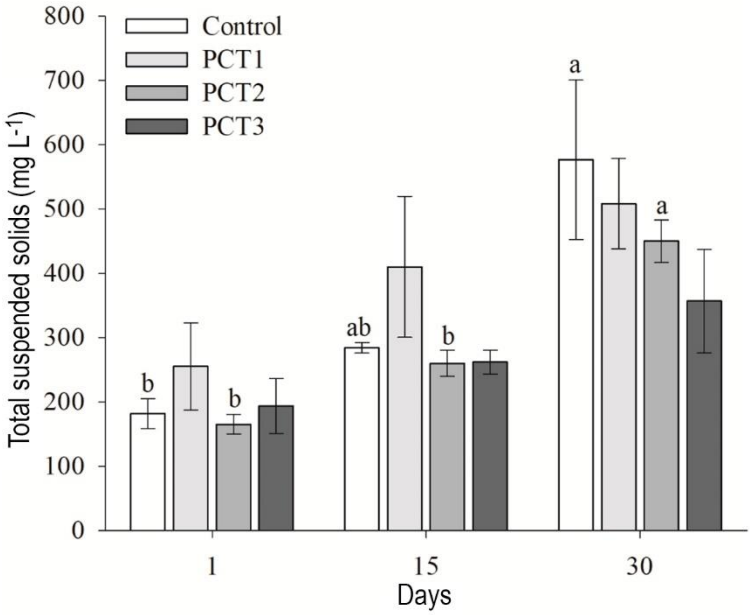

Figure 1. Mean total suspended solids concentration values (± standard error) during production of *P. vannamei* in biofloc system with probiotic consortium technology (PCT) inclusion. Different letters indicate significant differences within each treatment during the trial period (α = 5%).

Enzymatic activity evaluation

The values of trypsin, chymotrypsin, amylase, lipase, protein, and triglycerides in hepatopancreas, used as indicators of digestive enzyme activity, are presented (Table 4).

Mean values of trypsin and triglycerides did not differ significantly among treatments ($P > 0.05$; Table 4). For amylase and chymotrypsin, the mean values observed at PCT2 and PCT3 were higher than at control and PCT1. For lipase, the lowest values were observed at control and PCT1, which did not differ significantly from each other but were different for PCT2 and PCT3, the second being the highest value among treatments ($P < 0.05$; Table 4).

DISCUSSION

The highest concentrations of total ammonia (0.2108 mg L^{-1}) and nitrite (0.1275 mg L^{-1}) were within the safety level established for juveniles of *P. vannamei* in salinity between 25 and 35 (Lin & Chen 2001, 2003). Therefore, these variables did not influence shrimp performance.

The pH values observed are above the recommended for shrimp and outside the recommended range for *P. vannamei* (Van-Wyk et al. 1999). However, it does not appear to have affected the performance of the animals. Considering that there was good survival and there was no difference in pH among the treatments,

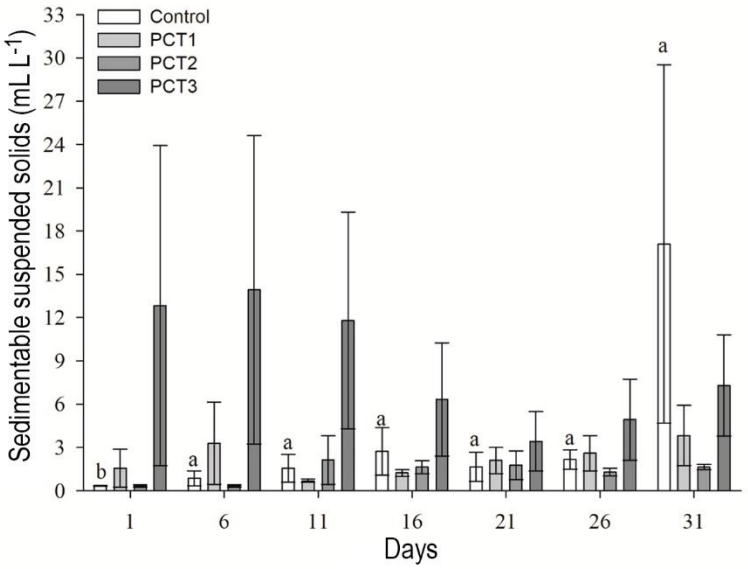

Figure 2. Mean sedimentable suspended solids concentration values (± standard error) during the production of *P. vannamei* in a biofloc system with probiotic consortium technology (PCT) inclusion. Different letters indicate significant differences within each treatment over the trial period (α = 5%).

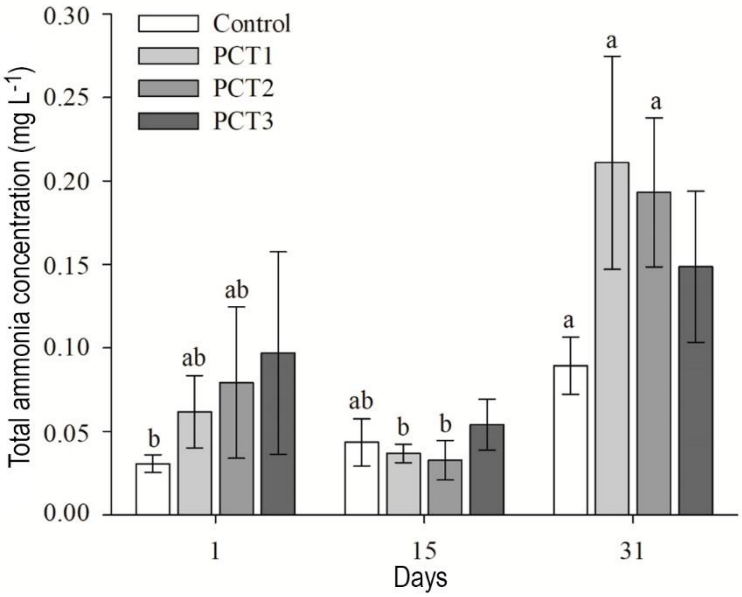

Figure 3. Mean total ammonia concentration values (± standard error) during the production of *P. vannamei* in a biofloc system with probiotic consortium technology (PCT) inclusion. Different letters indicate significant differences within each treatment over the trial period (α = 5%).

However, the toxicity of ammonia to aquatic organisms is directly proportional to the pH value: the more alkaline, the greater the proportion of toxic ammonia (non-ionized form), influenced by temperature (Van-Wyk et al. 1999, Randall & Tsui 2002, Kathyayani et al. 2019). According to Van-Wyk et al. (1999), it is recommended that the amount of toxic ammonia does not exceed 0.03 mg L^{-1}. The highest values of toxic ammonia were observed at PCT2 (0.035 mg L^{-1}) and PCT3 (0.083 mg L^{-1}) in the last week (total ammonia, PCT2 - 0.193 mg L^{-1}, PCT3 - 0.1486 mg L^{-1}). In the remaining weeks, the proportion of toxic ammonia only exceeded this limit for PCT2 (approximately 0.039 mg L^{-1}) and PCT3 (approximately 0.032 mg L^{-1}) in the first collection. Even with the highest values of toxic ammonia (no statistical differences), the performance

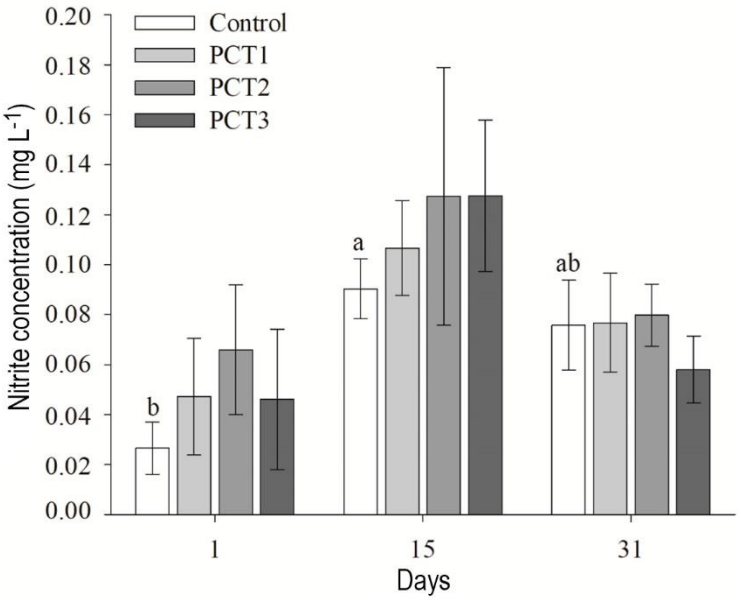

Figure 4. Mean nitrite concentration values (± standard error) during the production of *P. vannamei* in biofloc system with probiotic consortium technology (PCT) inclusion. Different letters indicate significant differences within each treatment over the trial period (α = 5%).

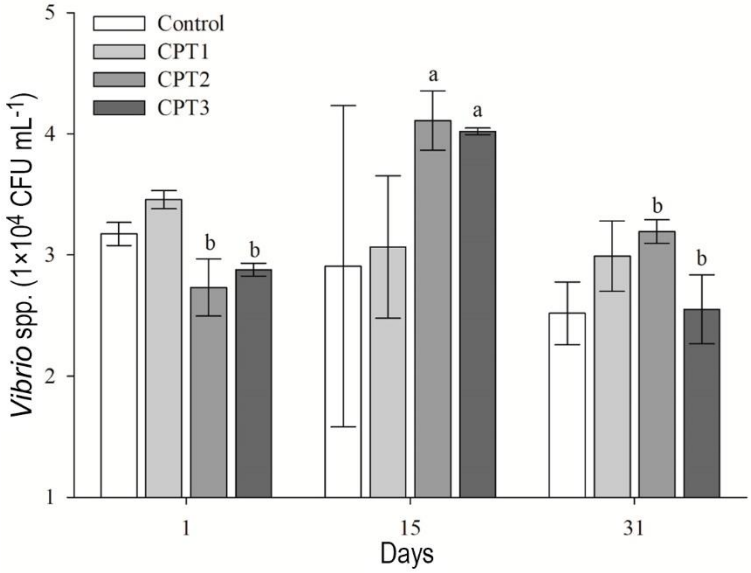

Figure 5. Mean values of CFU *Vibrio* spp. (± standard error) in water samples during the production of *P. vannamei* in a biofloc system. Different letters indicate significant differences within each treatment over the trial period (α = 5%).

of the animals was better in the treatments with probiotics.

Regarding TSS, Gaona et al. (2016) observed that values from 250 to 4,000 mg L^{-1} do not seem to affect the performance of *P. vannamei* when dissolved oxygen concentrations are maintained above 5 mg L^{-1}. Based on this information, it is possible to state that the TSS concentrations observed in this experiment (between 165 and 576.7 mg L^{-1}) did not influence the performance of shrimp. Although at control, PCT1, and PCT2, the average concentration of dissolved oxygen was below 5 mg L^{-1}, the values were very close, with the lowest average being of dissolved oxygen, 4.94 mg L^{-1}.

The probiotic did not promote significant differences in the other water quality variables, suggesting

Table 3. Mean values (±standard deviation) of the zootechnical variables of *P. vannamei* during production in a biofloc system with probiotic administration. Different superscripts on the same line indicate a significant difference among treatments ($P < 0.05$). FW: final weight; FL: final length; WG: weight gain; S: survival; FB: final biomass; BG: biomass gain; FCR: feed conversion rate; RWGR: relative weight growth rate; RLGR: relative length growth rate; SWGR: specific weight growth rate; SLGR: specific length growth rate, PCT: probiotic consortium technology.

Variables	Control	PCT1	PCT2	PCT3
FW (g)	8.15 ± 0.32	8.10 ± 0.11	8.67 ± 0.35	8.16 ± 0.57
FL (cm)	10.77 ± 0.12	10.70 ± 0.17	10.93 ± 0.06	10.63 ± 0.25
WG (g)	5.10 ± 0.32	5.05 ± 0.11	5.62 ± 0.35	5.11 ± 0.57
S (%)	84.60 ± 4.13	92.70 ± 3.12	90.00 ± 9.49	94.50 ± 9.36
FB (g)	254.94 ± 2.81^{b}	278.02 ± 11.49^{ab}	288.29 ± 18.67^{a}	284.18 ± 10.15^{ab}
BG (g)	159.37 ± 2.95^{b}	173.31 ± 8.19^{a}	186.63 ± 8.09^{a}	177.43 ± 3.47^{a}
FCR (g g^{-1})	1.41 ± 0.03^{a}	1.30 ± 0.06^{ab}	1.21 ± 0.05^{b}	1.27 ± 0.03^{b}
RWGR (%)	267.10 ± 10.60	265.46 ± 3.51	284.37 ± 1.52	267.43 ± 18.78
RLGR (%)	299.67 ± 11.55	293.00 ± 17.32	316.33 ± 5.77	286.33 ± 25.17
SWGR (%)	3.27 ± 0.13	3.25 ± 0.04	3.48 ± 0.14	3.27 ± 0.23
SLGR (%)	1.09 ± 0.04	1.07 ± 0.05	1.14 ± 0.02	1.05 ± 0.08

Table 4. Mean values (± standard deviation) of indicators of digestive enzymatic activity in the hepatopancreas of *P. vannamei* produced in biofloc system with probiotic administration. Different superscripts in the same column indicate a significant difference among treatments ($P < 0.05$). PCT: probiotic consortium technology.

Enzymes	Treatments			
	Control	PCT1	PCT2	PCT3
Amylase (U/L mg^{-1} protein)	9.08 ± 0.88^{b}	9.02 ± 1.12^{b}	10.36 ± 1.48^{a}	11.35 ± 1.16^{a}
Chymotrypsin (µmol min mg^{-1} protein)	0.676 ± 0.057^{b}	0.651 ± 0.032^{b}	0.733 ± 0.051^{a}	0.752 ± 0.064^{a}
Lipase (log x) (U/L mg^{-1} protein)	0.972 ± 0.154^{c}	0.971 ± 0.171^{c}	1.213 ± 0.411^{b}	1.659 ± 0.068^{a}
Triglycerides (mg mg^{-1})	192.301 ± 36.444	191.196 ± 36.444	212.556 ± 44.152	199.043 ± 56.616
Trypsin (µmol min mg^{-1} protein)	$6.16\times10^{-3} \pm 1.56\times10^{-3}$	$6.93\times10^{-3} \pm 1.38\times10^{-3}$	$7.08\times10^{-3} \pm 1.67\times10^{-3}$	$7.30\times10^{-3} \pm 2.17\times10^{-3}$

that the microorganisms present in the bioflocs efficiently maintained the system's balance. This statement can be supported by other authors (Seixas-Filho 2003, Ferreira et al. 2017, Frozza et al. 2021), who also did not observe differences in water quality due to using probiotics in a biofloc system. Apart from pH, all water quality variables evaluated in this study were within the values recommended for the species (Van-Wyk et al. 1999, Rajkumar et al. 2016, Kumar et al. 2018).

The probiotic evaluated in this study mainly comprises lactic acid bacteria, yeasts, *B. subtilis*, *B. animalis*, *L. casei*, and *S. cerevisiae*. This variety of species that make up the probiotic aims to increase the diversity of microflora in the digestive tract, acting synergistically in promoting benefits for the development of shrimp. Wang et al. (2019) observed that the mixture of probiotics (*Lactobacillus pentosus*, *L. fermentum*, *B. subtilis*, and *S. cerevisiae*) was more efficient in the growth performance and health status of shrimp than the addition of these probiotics alone.

In aquaculture, the use of probiotics has been studied mainly as a food supplement, as it promotes several benefits; for example, the increase in the immune responses of *P. vannamei* through the elevation of important metabolites in the regulation of this system, e.g. inosine monophosphate, valine, and betaine (Huynh et al. 2018). From the supplementation with *Lactobacillus plantarum*, Sánchez-Ortiz et al. (2016) and Yu et al. (2020) observed a reduction in *P. vannamei* mortality caused by *Vibrio harveyi*. Souza et al. (2012) observed a decrease in *Vibrio* concentrations in water due to using probiotics in the nursery rearing of the pink shrimp *Farfantepenaeus brasiliensis*. In the present study, no differences in *Vibrio* concentrations were observed among treatments for water or hepatopancreas. However, it is valid to speculate that, with the presence of these pathogens in all treatments, those who used probiotics had a higher S rate.

In this study, the use of the probiotic promoted improvements in shrimp performance, with higher values of FB and BG and more efficient FCR, contrary

to what was reported by Ferreira et al. (2017), in which no influence was observed on animal performance due to the use of probiotics. Studies have shown that probiotics promote benefits to the immune system (Roomiani et al. 2018), the composition of the intestinal microbiota (Fan & Li 2019), digestive tract morphology, and digestive enzyme activity, which together reflect an increase in the zootechnical performance of shrimp (Steffens 1989, Amoah et al. 2019). Souza et al. (2012) obtained higher final weight and specific growth rate in treatments with the probiotic.

Amoah et al. (2019) observed improvements in growth performance, intestinal morphology, microflora, immune response, and disease confrontation in addition to those described in this study; with a longer duration (56 days), it was possible to obtain higher values of final weight and specific growth rate. Feed conversion also improved; however, the values obtained in this study were more efficient, including control. In this experiment, the differences in performance were not manifested in individual variables but in collective ones. In addition, the experiment time (approximately 4.5 weeks) was short compared to the usual production time (13 to 20 weeks; Ruiz-Velazco et al. 2021). The short experimental period was intended to identify whether it was possible to observe any positive result from the probiotic effect in a few weeks since the studies in literature prioritize a long-term effect. Even so, with a short-term experiment, it was possible to observe positive results.

The absence of some proteases, carbohydrases, and lipases observed in shrimp's digestive tract limits the absorption of nutrients contained in macromolecules, mainly of ingredients of plant origin. In this sense, using bacteria capable of producing these enzymes or stimulating endogenous production can make digestion more efficient (Olmos et al. 2020, Ringø et al. 2020). The action of chymotrypsin, trypsin, amylases, and lipases is crucial for greater efficiency in the use of the diet, as they are enzymes involved in the digestion of macromolecules such as proteins, carbohydrates, and lipids (Tsai et al. 2019, Munaeni et al. 2020, Nelson et al. 2022). Thus, in this study, the increase in the activity of these enzymes observed in treatments using probiotics was reflected in the performance improvement mentioned above. This statement can be supported based on the study by Zheng et al. (2018), in which they also observed an improvement in growth related to the enzymatic activity of *P. vannamei*. Tsai et al. (2019) also attributed the greater growth of *P. vannamei* to improved diet digestibility, associating this improvement with increased enzyme activity.

Trypsin and chymotrypsin are proteases that act on the hydrolysis of peptides, presenting distinct specificities regarding the amino acids they act, which are essential for protein digestion (Nelson et al. 2022). Chymotrypsin values were significantly higher in treatments with the addition of probiotics and trypsin (although this second does not present a significant difference), indicating greater efficiency in the digestion of this macronutrient. In the study by Liu et al. (2009), an isolate of *B. subtilis* proved to be a major producer of protease, capable of improving shrimp performance by increasing food digestibility.

The natural microorganisms of the biofloc promote several benefits to the production environment and animals, cycling nutrients and being able to serve as food, in addition to assisting in resistance against pathogens (Aalimahmoudi et al. 2017, Emerenciano et al. 2017, Almeida et al. 2021). The use of the probiotic PCT to enhance the natural benefits of the biofloc was able to improve the performance of *P. vannamei* juveniles, as mentioned above, promoting gains in biomass and improved feed conversion rate, which is one of the factors that most influence the net revenue of shrimp farms (Ruiz-Velazco et al. 2021).

CONCLUSIONS

The use of probiotics promotes increased digestive enzymatic activity, providing greater diet efficiency. This consequently benefits the zootechnical performance of *P. vannamei* regarding final biomass, biomass gain, and feed conversion. These performance indicators are factors that directly influence the profitability of shrimp farming in all phases, especially in the grow-out phase.

Better results were obtained when PCT was administered daily, at doses of 100 mL 100,000 L^{-1} in water and 3 mL kg^{-1} of feed in the diet (PCT2), during the initial fattening phase of *P. vannamei* in a biofloc system.

Data availability

Data will be made available on request.

Credit author contribution

A. Faveta & C.H.N. Ferreira: Conceptualization, project administration, methodology, validation, data curation, formal analysis, writing-original draft; F.M. Dutra: Supervision, statistical analysis, writing-original draft, review, editing and formal analysis; L.H. Cazarolli: Methodology, enzyme analysis, review and

editing; E.L.C. Ballester: Funding acquisition, conceptualization, formal analysis, review, and editing. All authors have read and accepted the published version of the manuscript.

Ethical approval

Studies with invertebrates do not require authorization from the Ethics and Animal Welfare Committee of the Federal University of Paraná, Brazil. However, the procedures value animal welfare, following the recommendations of the Animal Behavior Association (ASAB, 2003), the UK use of animals for scientific purposes law (Scientific Procedures Act, 1986) and associated guidelines, and EU Directive 2010/63/EU for animal experiments.

Conflict of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest. The funding source had no role in this study's design, practice, or analysis.

ACKNOWLEDGMENTS

The authors thank the Experimental Nucleus of Applied Mycology (UFPR) and Doctor Professor Adriana Fiorini for supporting Vibrio analyses. We thank the Biochemistry and Genetics Laboratory (UFFS) and Doctor Professor Luisa Helena Casarolli for their support in enzymatic analysis. A. Favetta and C.H.N. Ferreira, thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES; Finance Code 001) for the study scholarships. E.L.C. Ballester (PQ process: 311456/2020-0) received support from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the researcher productivity grant. F.M. Dutra (PDJ process: 151148/2022-8) received support from the Council for Scientific and Technological Development (CNPq) for the study scholarships.

REFERENCES

Aalimahmoudi, M., Azarm, H.M. & Mohamadi, Y. 2017. Biofloc new technology and shrimp disease in super-intensive aquaculture. International Journal of Fisheries and Aquatic Research, 2: 17-21.

Adel, M., El-Sayed, A.F.M., Yeganeh, S., Dadar, M. & Giri, S.S. 2017. Effect of potential probiotic *Lactococcus lactis* subsp. lactis on growth performance, intestinal microbiota, digestive enzyme activities, and disease resistance of *Litopenaeus vannamei*. Probiotics and Antimicrobial Proteins, 9: 150-156. doi: 10.1007/s12602-016-9235-9

Almeida, M.S., Carrijo-Mauad, J.R., Gimenes, R.M.T., Gaona, C.A.P., Furtado, P.S., Poersch, L.H., et al. 2021. Bioeconomic analysis of the production of marine shrimp in greenhouses using the biofloc technology system. Aquaculture International, 29: 723-741. doi: 10.1007/s10499-021-00653-1

Amoah, K., Huang, Q.C., Tan, B.P., Zhang, S., Chi, S.Y., Yang, Q.H., et al. 2019. Dietary supplementation of probiotic *Bacillus coagulans* ATCC 7050 improves the growth performance, intestinal morphology, microflora, immune response, and disease confrontation of Pacific white shrimp, *Litopenaeus vannamei*. Fish and Shellfish Immunology, 87: 796-808. doi: 10.1016/j. fsi.2019.02.029

Antunes, C.R.N., Ledo, C.A.D.S., Pereira, C.M. & Santos, J.D. 2018. Evaluation of feeding rates in the production of *Litopenaeus vannamei* shrimp using artificial substrates. Ciência Animal Brasileira, 19: 19. doi: 10.1590/1809-6891v19e-50805

Arshad, F.A., Mehmood, R., Hussain, S., Khan, M.A. & Khan, M.S. 2018. Lactobacilli as probiotics and their isolation from different sources. British Journal of Research, 5: 1-11. doi: 10.21767/2394-3718.100043

Avnimelech, Y. 2009. Biofloc technology: a practical guidebook. World Aquaculture Society, Baton Rouge.

Baird, R.B., Eaton, A.D. & Clesceri, L.S. 2012. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC.

Bautista-Teruel, M.N., Eusebio, P.S. & Welsh, T.P. 2003. Utilization of feed pea, *Pisum sativum*, meal as a protein source in practical diets for juvenile tiger shrimp, *Penaeus monodon*. Aquaculture, 225: 121-131. doi: 10.1016/S0044-8486(03)00284-9

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-254. doi: 10.1016/0003-2697(76)90527-3

Carrillo-Farnés, O., Forrellat-Barrios, A., Guerrero-Galván, S. & Vega-Villasante, F. 2007. A review of digestive enzyme activity in penaeid shrimps. Crustaceana, 80: 257-275.

Castellone, V. 2022. Lactic acid bacteria and bioactive compounds: production in fermented foods and fate after digestion. Ph.D. Thesis, Universita' Degli Studi di Parma, Parma.

Emerenciano, M., Gaxiola, G. & Cuzon, G. 2013. Biofloc technology (BFT): a review for aquaculture application and animal food industry. Biomass Now-Cultivation and Utilization, 12: 301-328. doi: 10.5772/53902

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. & Gruver, B.P. (Eds.). Water quality. IntechOpen, London, pp. 92-109.

Fan, L. & Li, Q.X. 2019. Characteristics of intestinal microbiota in the Pacific white shrimp *Litopenaeus vannamei* differing growth performances in the marine cultured environment. Aquaculture, 505: 450-461. doi: 10.1016/j.aquaculture.2019.02.075

Fathima, S., Shanmugasundaram, R., Adams, D. & Selvaraj, R.K. 2022. Gastrointestinal microbiota and their manipulation for improved growth and performance in chickens. Foods, 11: 1-30. doi: 10.3390/foods11101401

Ferreira, M.G.P., Melo, F.P., Lima, J.P.V., Andrade, H.A., Severi, W. & Correia, E.S. 2017. Bioremediation and biocontrol of commercial probiotic in marine shrimp culture with biofloc. Latin American Journal of Aquatic Research, 45: 167-176. doi: 10.3856/vol45-issue1-fulltext-16

Food and Agriculture Organization (FAO). 2022. The state of world fisheries and aquaculture: Towards blue transformation. FAO, Rome.

Frozza, A., Fiorini, A., Vendruscolo, E.C.G., Rosado, F.R., Konrad, D., Rodríguez, M.C.G., et al. 2021. Probiotics in the rearing of freshwater prawn *Macrobrachium rosenbergii* (de Man, 1879) in a biofloc system. Aquaculture Research, 52: 4269-4277. doi: 10.1111/are.15265

Fuller, R. 1989. Probiotics in man and animals. Journal of Applied Bacteriology, 66: 365-378. doi: 10.1111/j.1365-2672.1989.tb05105.x

Gaona, C.A.P., da Paz Serra, F., Furtado, P.S., Poersch, L.H. & Wasielesky Jr., W. 2016. Effect of different total suspended solids concentrations on the growth performance of *Litopenaeus vannamei* in a BFT system. Aquacultural Engineering, 72: 65-69. doi: 10.1016/j.aquaeng.2016.03.004

George, E.G.J., Jayaraj, G.P., Balaraman, D. & Soundarapandy, A. 2016. Augmenting efficacy of the commercial probiotic consortium, Ecotrax® on soil, water quality, survival, growth, and feed transformation on the semi-intensive pond culture system of the white leg shrimp, *Litopenaeus vannamei* (Boone, 1931). Advances in Applied Science Research, 7: 32-42.

Hummel, B.C. 1959. A modified spectrophotometric determination of chymotrypsin, trypsin, and thrombin. Canadian Journal of Biochemistry and Physiology, 37: 1393-1399. doi: 10.1139/o59-157

Huynh, T.G., Cheng, A.C., Chi, C.C., Chiu, K.H. & Liu, C.H. 2018. A symbiotic improves the immunity of white shrimp, *Litopenaeus vannamei*: Metabolomic analysis reveals compelling evidence. Fish and Shellfish Immunology, 79: 284-293. doi: 10.1016/j.fsi.2018.05.031

Jiménez-Ordaz, F.J., Cadena-Roa, M.A., Pacheco-Vega, J.M., Rojas-Contreras, M., Tovar-Ramírez, D. & Arce-Amezquita, P.M. 2021. Microalgae and probiotic bacteria as biofloc inducers in a hyper-intensive Pacific white shrimp (*Penaeus vannamei*) culture. Latin American Journal of Aquatic Research, 49: 155-168. doi: 10.3856/vol49-issue1-fulltext-2442

Kathyayani, S.A., Poornima, M., Sukumaran, S., Nagavel, A. & Muralidhar, M. 2019. Effect of ammonia stress on immune variables of Pacific white shrimp *Penaeus vannamei* under varying levels of pH and susceptibility to white spot syndrome virus. Ecotoxicology and Environmental Safety, 184: 109626. doi: 10.1016/j.ecoenv.2019.109626

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 and Shellfish Immunology, 94: 175-189. doi: 10.1016/j.fsi.2019.09.013

Kumar, V.S., Pandey, P.K., Anand, T., Bhuvaneswari, G.R., Dhinakaran, A. & Kumar, S. 2018. Biofloc improves water, effluent quality, and growth parameters of *Penaeus vannamei* in an intensive culture system. Journal of Environmental Management, 215: 206-215. doi: 10.1016/j.jenvman.2018.03.015

Kumar, S.D., Santhanam, P., Krishnaveni, N., Raju, P., Begum, A., Ahmed, S.U., et al. 2020. Baseline assessment of water quality and ecological indicators in *Penaeus vannamei* farm wastewater along the Southeast coast of India. Marine Pollution Bulletin, 160: 111579. doi: 10.1016/j.marpolbul.2020.111579

Lara, G., Hostins, B., Bezerra, A., Poersch, L. & Wasielesky Jr., W. 2017. The effects of different feeding rates and re-feeding of *Litopenaeus vannamei* in a biofloc culture system. Aquacultural Engineering, 77: 20-26. doi: 10.1016/j.aquaeng.2017.02.003

Lin, Y.C. & Chen, J.C. 2001. Acute toxicity of ammonia on *Litopenaeus vannamei* Boone juveniles at different salinity levels. Journal of Experimental Marine Biology and Ecology, 259: 109-119. doi: 10.1016/S0022-0981(01)00227-1

Lin, Y.C. & Chen, J.C. 2003. Acute toxicity of nitrite on *Litopenaeus vannamei* (Boone) juveniles at different salinity levels. Aquaculture, 224: 193-201. doi: 10.1016/S0044-8486(03)00220-5

Liu, C.H., Chiu, C.S., Ho, P.L. & Wang, S.W. 2009. Improvement in the growth performance of white shrimp, *Litopenaeus vannamei*, by a protease-producing probiotic, *Bacillus subtilis* E20, from natto. Journal of Applied Microbiology, 107: 1031-1041. doi: 10.1111/j.1365-2672.2009.04284.x

Llario, F., Falco, S., Sebastiá-Frasquet, M.T., Escrivá, J., Rodilla, M. & Poersch, L.H. 2019. The role of *Bacillus amyloliquefaciens* on *Litopenaeus vannamei* during the maturation of a biofloc system. Journal of Marine Science and Engineering, 7: 228. doi: 10.3390/jmse7070228

Moreira, G.M., Meneses, J.A.M., Ribeiro, C.V., Faria, A.D.M., Arantes, H.G., Luz, M.H.D., et al. 2019. Performance and feed efficiency of beef cattle fed high energy diet with probiotic consortium technology. Revista Brasileira de Saúde e Produção Animal, 20: 1-13. doi: 10.1590/S1519-9940200182019

Munaeni, W., Yuhana, M., Setiawati, M. & Wahyudi, A.T. 2020. Impact of dietary supplementation with *Eleutherine bulbosa* (Mill.) Urb. on intestinal microbiota diversity and growth of white shrimp, *Litopenaeus vannamei*. Aquaculture, 528: 735466. doi: 10.1016/j.aquaculture.2020.735466

Nelson, D.L., Cox, M.M. & Hoskins, A.A. 2022. Princípios de bioquímica de Lehninger. Artmed, Porto Alegre.

Niu, J., Wen, H., Li, C.H., Liu, Y.J., Tian, L.X., Chen, X.U., et al. 2014. Comparison effect of dietary astaxanthin and β-carotene in the presence and absence of cholesterol supplementation on growth performance, antioxidant capacity, and gene expression of *Penaeus monodon* under normoxia and hypoxia condition. Aquaculture, 422: 8-17. doi: 10.1016/j.aquaculture.2013.11.013

Olmos, J., Acosta, M., Mendoza, G. & Pitones, V. 2020. *Bacillus subtilis*, an ideal probiotic bacterium to shrimp and fish aquaculture that increase feed digestibility, prevent microbial diseases, and avoid water pollution. Archives of Microbiology, 202: 427-435. doi: 10.1007/s00203-019-01757-2

Peixoto, S., Silva, E., Costa, C.B., Nery, R.C., Rodrigues, F., Silva, J.F., et al. 2018. Effect of feeding frequency on growth and enzymatic activity of *Litopenaeus vannamei* during nursery phase in biofloc system. Aquaculture Nutrition, 24: 579-585. doi: 10.1111/anu.12591

Rajkumar, M., Pandey, P.K., Aravind, R., Vennila, A., Bharti, V. & Purushothaman, C.S. 2016. Effect of different biofloc systems on water quality, biofloc composition, and growth performance in *Litopenaeus vannamei* (Boone, 1931). Aquaculture Research, 47: 3432-3444. doi: 10.1111/are.12792

Randall, D.J. & Tsui, T.K.N. 2002. Ammonia toxicity in fish. Marine Pollution Bulletin, 45: 17-23. doi: 10.1016/S0025-326X(02)00227-8

Ringø, E., van Doan, H., Lee, S.H., Soltani, M., Hoseinifar, S.H., Harikrishnan, R., et al. 2020. Probiotics, lactic acid bacteria, and bacilli: interesting supplementation for aquaculture. Journal of Applied Microbiology, 129: 116-136. doi: 10.1111/jam.14628

Rocha, J.V., Silva, J.F., Barros, C., Peixoto, S. & Soares, R. 2019. Compensatory growth and digestive enzyme activity of *Litopenaeus vannamei* submitted to feeding restriction in a biofloc system. Aquaculture Research, 50: 3653-3662. doi: 10.1111/are.14323

Roomiani, L., Ahmadi, S. & Ghaeni, M. 2018. Immune response and disease resistance in the white shrimp, *Litopenaeus vannamei* induced by potential probiotic *Lactobacillus*. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 65: 323-329. doi: 10.1501/Vetfak_0000002863

Rosado, P.M., Cardoso, P.M., Rosado, J.G., Schultz, J., Nunes da Rocha, U., Keller-Costa, T., et al. 2023. Exploring the potential molecular mechanisms of interactions between a probiotic consortium and its coral host. mSystems, 8: e00921-22. doi: 10.1128/msystems.00921-22

Ruiz-Velazco, J.M., González-Romero, M.A., Estrada-Perez, N. & Hernandez-Llamas, A. 2021. Evaluating partial harvesting strategies for whiteleg shrimp *Litopenaeus* (*Penaeus*) *vannamei* semi-intensive commercial production: profitability, uncertainty, and economic risk. Aquaculture International, 29: 1317-1329. doi: 10.1007/s10499-021-00695-5

Sánchez-Ortiz, A.C., Angulo, C., Luna-González, A., Álvarez-Ruiz, P., Mazón-Suástegui, J.M. & Campa-Córdova, A.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

and Shellfish Immunology, 59: 95-102. doi: 10.1016/j.fsi.2016.10.022

Seixas-Filho, J.T. 2003. Revisão sobre as enzimas digestivas nos peixes teleostei e seus métodos de determinação. Augustus, 17: 30-45.

Shanmugasundaram, S., Mayavu, P., Manikandarajan, T., Suriva, M., Enswar, A. & Anbarasu, R. 2015. Isolation and identification of *Vibrio* sp. in the hepatopancreas of cultured white Pacific shrimp (*Litopenaeus vannamei*). International Letters of Natural Sciences, 46: 52-59. doi: 10.18052/www.scipress.com/ILNS.46.52

Soto-Rodriguez, S.A., Gomez-Gil, B., Lozano-Olvera, R., Betancourt-Lozano, M. & Morales-Covarrubias, M.S. 2015. Field and experimental evidence of *Vibrio* parahaemolyticus as the causative agent of acute hepatopancreatic necrosis disease of cultured shrimp (*Litopenaeus vannamei*) in northwestern Mexico. Applied and Environmental Microbiology, 81: 1689-1699. doi: 10.1128/AEM.03610-14x

Souza, D.M., Suita, S.M., Leite, F.P.L., Romano, L.A., Wasielesky, W. & Ballester, E.L.C. 2012. The use of probiotics during the nursery rearing of the pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817) in a zero exchange system. Aquaculture Research, 43: 1828-1837. doi: 10.1111/j.1365-2109.2011.02992.x

Steffens, W. 1989. Principles of fish nutrition. Ellis Horwood Limited, Chichester.

Tsai, C.Y., Chi, C.C. & Liu, C.H. 2019. The growth and apparent digestibility of white shrimp, *Litopenaeus vannamei*, are increased with the probiotic *Bacillus subtilis*. Aquaculture Research, 50: 1475-1481. doi: 10.1111/are.14022

Tuan, T.N., Duc, P.M. & Hatai, K. 2013. Overview of the use of probiotics in aquaculture. International Journal of Research in Fisheries and Aquaculture, 3: 89-97.

Van Wyk, P., Davis-Hodgkins, M., Laramore, R., Main, K.L., Mountain, J. & Scarpa, J. 1999. Farming marine shrimp in recirculating freshwater systems. Harbor Branch Oceanographic Institution, Florida.

Vieira, F.N., Buglione, C.C., Mourino, J.P.L., Jatobá, A., Martins, M.L., Schleder, D.D., et al. 2010. Effect of probiotic supplemented diet on marine shrimp survival after challenge with *Vibrio harveyi*. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 62: 631-638. doi: 10.1590/S0102-09352010000300019x

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 and Shellfish Immunology, 84: 1050-1058. doi: 10.1016/j.fsi.2018.11.017

Xu, W., Xu, Y., Su, H., Hu, X., Xu, Y., Li, Z., et al. 2021. Production performance, inorganic nitrogen control, and bacterial community characteristics in a controlled biofloc-based system for indoor and outdoor super-intensive culture of *Litopenaeus vannamei*. Aquaculture, 531: 735749. doi: 10.1016/j.aquaculture.2020.735749

Yu, P., Wang, T., Ye, H., Shan, H. & Ma, S. 2020. Isolation and identification of pathogenic *Vibrio* spp. retrieved from diseased *Litopenaeus vannamei* and beneficial role of some functional probiotic bacteria for control. Aquaculture International, 28: 1403-1420. doi: 10.1007/s10499-020-00530-3

Zheng, X., Duan, Y., Dong, H. & Zhang, J. 2018. Effects of dietary *Lactobacillus plantarum* on growth performance, digestive enzymes and gut morphology of *Litopenaeus vannamei* Probiotics and Antimicrobial Proteins, 10: 504-510. doi: 10.1007/s12602-017-9300-z

Received: October 17, 2023; Accepted: August 15, 2024