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Different strategies of use of commercial effective microorganisms in the rearing of white shrimp *Litopenaeus vannamei* in biofloc system

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ABSTRACT. An experiment was conducted for eight weeks, in a BFT system, to test different strategies for using effective microorganisms (AEM) in shrimp rearing. For that, five different treatments were tested. The treatments tested were: Control (commercial feed only), AEM in the feed (addition of AEM in the commercial feed), AEM in the water (addition of AEM directly in the rearing water), AEM in the feed and in the water (addition of AEM in the feed simultaneously with the addition of AEM in the water) and MA in the feed (addition of the microbiological activator-MA in the commercial feed, without adding microorganisms to the feed and water). The use of AEM or MA, although it did not affect the growth of the animals, stimulated the digestive process of the animals, allowing the required absorption of nutrients with a smaller intestinal area. Associated with this, the presence of AME or MA stimulated the immunological parameters of the animals and reduced the presence of *Vibrio* in the gut of the shrimp. The association of these factors meant that the survival of the animals that received the additives in the diet was substantially higher than the animals that did not receive the additives. The AEM supplementation strategies did not differ among themselves, and the producer could opt for the strategy of lower cost and/or less complexity of use, according to each specific case.

Keywords: probiotic; immunology; histomorphometry; bioremediation; *Vibrio*.

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Introduction

The shrimps rearing has advanced to found alternatives more environment-friendly, combining productivity, expansion, minimum environmental impact, and minimum manufacturing of wastes consisting of extra nutrients, poisonous compounds, and pathogens. These structures use biofloc technology (BFT), which allows for the degradation of natural waste and the assimilation and nitrification of nitrogen with the aid of a huge microbial network that shapes the biofloc under extreme aeration and mixing. The biofloc accommodates diverse microorganisms, uneaten feed, useless cells, detritus, and feces. In a BFT system, an extra supply of natural carbon under a controlled ratio of carbon to nitrogen (C:N) is used to compensate for every increase and multiplication of microorganisms within the water culture. The microorganism then maintains the desired water parameters and serves as a supplementary supply of proteins, lipids, vitamins, and minerals for the raised animals (Ferreira et al., 2017).

Another method to achieve sustainable aquaculture has been the use of microorganisms with a probiotic and/or bioremediation function as an alternative to antibiotics. Probiotics in aquaculture are cultured products or living microbial supplements that have beneficial effects on the hosts by altering their intestinal microbial community. Potential benefits of probiotics include improved water quality, better nutrition of the host species through the production of additional digestive enzymes, lower incidence of disease, increased survival, and better immune response (Zhou, Wang, & Li, 2009). Probiotic species are often isolated from shrimp guts and the surrounding water or sediments of their environment. However, they have also been isolated from fruit pulp filtrates, curd, fermented soybeans, fermented pickles, and viscera of other species (Knipe, Temperton, Lange, Bass, & Tyler, 2021).

However, for the effective use of probiotics, parameters such as time, dosage, routes of administration and physiological conditions must be carefully considered. Probiotics should be introduced into cropping systems before a disease outbreak in order to reduce disease-related losses. There are several ways to introduce probiotics to shrimp, such as feed supplement, soaking and oral administration. However, feeding or feed supplementation has proven to be more efficient and practical than others, since most probiotics are designed to be mixed with food. Oral administration is considered the most practical route for shrimp probiotics and its use is beneficial to shrimp regardless of animal size, as shrimp can be treated at any stage of the growing season. A suitable probiotic density is 10^5 CFU mL^{-1} (van Hai & Fotedar, 2010).

The microbial species composition of aquaculture systems can be modified through the inclusion of strategic species to compete with and control dangerous microorganisms. Success relies on defining the ecological strategies to be altered, the types of pathogenic species which are dominant, and the acceptable opportunity species of microorganism that may be added. Competitive exclusion is one of the ecological strategies that permits the manipulation of bacterial species composition in water, sediments, and animal viscera (Moriarty, 1999).

This study was conducted to investigate changes in water quality and shrimp production in white shrimp ponds, *Litopenaeus vannamei,* through different commercial probiotic use strategies.

Materials and methods

Probiotic product

A commercial product was used to test the effect of efficient microorganisms (EM) under different usage strategy on shrimp rearing. The tested product was Embiotic (Korin, Brazil). According to the manufacturer's specifications, they contained 1.3×10^4 colony forming units (CFU) of *Saccharomyces cerevisiae* mL^{-1}, and 3.3×10^4 CFU of Bacilli mL^{-1}. The product was activated (AEM - activated EM) by fermentation under anaerobic conditions in water with a commercial microbiological activator MA (Hibana - Korin, Brazil) for seven days until pH was below 3.5. The Final concentration of microorganisms after seven days of fermentation were 2.6×10^6 CFU mL^{-1} of *Saccharomyces cerevisiae*, and 3.3×10^8 CFU of Bacilli mL^{-1}.

Growth assay

An experiment was conducted for eight weeks, in a BFT system, to test different strategies for using AEM in shrimp rearing. For that, five different treatments were tested. The treatments tested were: Control (commercial feed only), AEM in the feed (addition of AEM in the commercial feed), AEM in the water (addition of AEM directly in the rearing water), AEM in the feed and in the water (addition of AEM in the feed simultaneously with the addition of AEM in the water), and MA in the feed (addition of the microbiological activator-MA in the commercial feed, without adding microorganisms to the feed and water). Table 1 presents the composition of the treatments with their respective concentrations of use of the activated solutions.

Table 1. Summary and concentrations of the activated solutions of microorganisms or activator solution used in each treatment for rearing shrimp *Litopenaeus vannamei* fed with different strategies of AEM during eight weeks in BFT system.

Treatment	AEM Feed (mL kg^{-1})	AEM Water (mL L^{-1})	MA Feed (mL kg^{-1})
Control	0.0	0.0	0.0
AEM feed (AEM-F)	50.0	0.0	0.0
AEM water (AEM-W)	0.0	0.5	0.0
AEM feed and water (AEM-FW)	50.0	0.5	0.0
MA feed (MA-F)	0.0	0.0	50.0

The probiotic (EM) and microbiological activator (MA) activations were performed one week before the start of the experiment and repeated every seven days throughout the experimental period. The activation of the probiotic (AEM) was done using 50 mL of the inactivated product (Embiotic) and 1,500 g of the microbiological activator (Hibana) in a bucket with 10 L of filtered water, with salinity of 1.0 ppt. The activated solution used in the MA-F treatment contained 1,500 g of the microbiological activator (Hibana) in a bucket with 10 L of filtered water, with salinity of 1.0 ppt, without the addition of the commercial probiotic. In the treatments with the addition of activated solutions (liquid) in the diet, the activated solutions were daily

added to the feed by spraying, 30 min. before feeding. In the treatments with probiotic in the water, the AEM was applied daily, through the direct release in the rearing water

The experimental units consisted of circular tanks of 800 L useful volume, individually equipped with microperforated aeration hoses connected to an air blower and heaters with temperature control (28.5 ± 0.1ºC). Each tank was stocked with 200 shrimp (density of 250 shrimp m^{-3}) with an initial weight of 3.40 ± 0.01 g. Shrimp were fed with 1.6 mm commercial extruded feed, according to the feeding table proposed by Van Wyk et al. (1999), four times a day (08:00 a.m., 11:00 a.m., 02:00 p.m., and 05:00 p.m.) in feeding trays. Feeding was corrected weekly, after each weekly biometry. The centesimal composition of the diet was: crude protein (350 g kg^{-1}), ether extract (75 g kg^{-1}), crude fiber (40 g kg^{-1}), mineral matter (140 g kg^{-1}), moisture (100 g kg^{-1}) and did not have any probiotics in its basic composition. At the end of the experiment, all animals were weighed to evaluate zootechnical indexes.

Gut histology

At the end of the rearing period, a one-centimeter transverse fragment from the second abdominal segment was collected from each of four shrimps sampled from each tank (12 per treatment), which were then immediately fixed in Davidson's fixative solution for 24 hours. Tissues were then subjected to serial dehydration steps in alcohol (30–100%) and embedded in paraffin blocks. Sections with 4.0 µm in thickness were stained with hematoxylin–eosin and examined under a light microscope (Zeiss, Axio Imager A.2, Gottingen, Germany), coupled to a photo documentation system (Canon PowerShot G9). The number, length, area and of folds were measured by Image Q Capture Pro 5.1 software.

Immunology parameters

Shrimp hemolymph (n = 20 for each group) was withdrawn from the shrimp's ventral sinus by inserting a needle (13 × 0.4 mm) coupled to a 1.0 mL syringe. For the preparation of the serum, the hemolymph collected (4 pools of five animals per group) was allowed to clot for two hours at room temperature. The clot formed was broken up with the aid of a glass rod and repeatedly centrifuged at 6,000 × g for 10 min. The supernatant or serum (plasma deprived of clot protein + exocytosed cell products) was collected and stored at −20°C for use on protein concentration and phenoloxidase activity tests.

Total hemocyte counts (THC) were estimated using a Neubauer chamber (Beçak & Paulete, 1976). Hemolymph (four pools of five animals per group) was collected in fixative composed of 4% formaldehyde in anticoagulant solution (336 mM NaCl, 115 mM glucose, 27 mM sodium citrate, 9.0 mM EDTA, pH 7.2) and was kept at 4.0 °C until analysis.

The Phenoloxidase enzyme activity in the serum samples (PO) was determined spectrophotometrically (A490) through formation of a red pigment (Dopa-chrome) by oxidation of the L-Dopa enzyme substrate. Serum samples were diluted (15×) in TBS 1 (10 mM Tris, 5 mM $MgCl_2$, 10 mM $CaCl_2$, 336 mM NaCl, pH 7.4) and 50 µL was pre-incubated with an equal volume of trypsin (Sigma) (1 mg mL^{-1}) for five min at room temperature, in 96-well microplate. For the controls, the inductor or the serum was replaced by TBS. Then, the wells received 50 µl of L-Dopa (3 mg mL^{-1}) and the formation of Dopa-chrome was measured (A490) in a microplate reader (Tecan, Barueri, SP) after 5, 10, 15 and 20 min. One unit of enzymatic activity was equivalent to a change of 0.001 in the absorbance $min.^{-1}$ mg^{-1} of protein at 20 °C (Söderhäll & Häll, 1984). All tests were performed in triplicate.

The serum protein content (four pools of five animals per group) was determined by the Bradford (1976), using bovine serum albumin (BSA) as standard. The tests were performed in triplicate.

Water quality

During the experiment, the dissolved oxygen and temperature were measured twice per day, at 08:00 a.m. and 05:00 p.m. (Dissolved Oxygen Meter YSI, model Pro20). The pH (pH meter YSI, modelpH100), total suspended solids, nitrate, alkalinity (American Public Health Association [Apha], 2005), total ammonia nitrogen (TAN), nitrite, orthophosphate (Strickland & Parsons, 1972), and salinity (EcoSense YSI, model EC300A) were measured twice per week.

Microbiology

Five shrimps from each experimental unit were sampled to form a pool per unit for microbiology analyses. The midguts of the shrimps were excised with forceps and scalpel, and then homogenized in sterile saline

solution in 3% of NaCl in a grail. The samples were then serially diluted (1/10) and seeded in Agar Marine culture medium for total heterotrophic marine bacteria, and in Agar TCBS (Thiosulphate Citrate Bile Sucrose), which is a selective medium for *Vibrio*. Finally, they were incubated at 29 °C for 24 hours.

Statistical analysis

Water quality variables were analyzed by one-way repeated measures ANOVA. Performance indexes, immunology parameters, histology, and microbiological data were compared by one-way ANOVA supplemented by the Tukey test of separation of averages. Water quality parameters were evaluated by one-way ANOVA to compare the mean of each treatment and repeated measure ANOVA to compare the parameters weekly for each treatment. Homoscedasticity and normality were tested by Bartlett and Shapiro-Wilk tests, respectively. All statistical tests were evaluated at a 5% significance level.

Results

The use of AEM directly affected the zootechnical parameters of shrimp. At the end of the eight weeks, the shrimp in the control group had a significantly lower survival rate than the other treatments (Figure 1), however, there was no difference among the AEM use strategies. In addition to survival, final biomass, and biomass gain (Figure 2A and 2B, respectively) were also lower in the control treatment. Following the survival behavior, the different AEM use strategies did not affect the results of final biomass and biomass gain ($p > 0.05$) in comparison with the other treatments (Figure 3). Additional zootechnical parameters were not affected using AEM or the use strategy (Table 2). The water quality parameters were not affected by AEM use strategy or absence of AEM (Table 3).

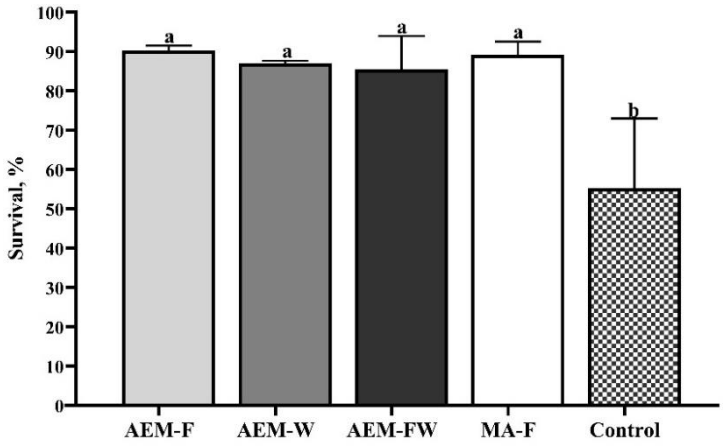

Figure 1. Survival of shrimp fed with different strategies of AEM during eight weeks in BFT system. Values presented as mean and SD. Different letters represents statistical difference.

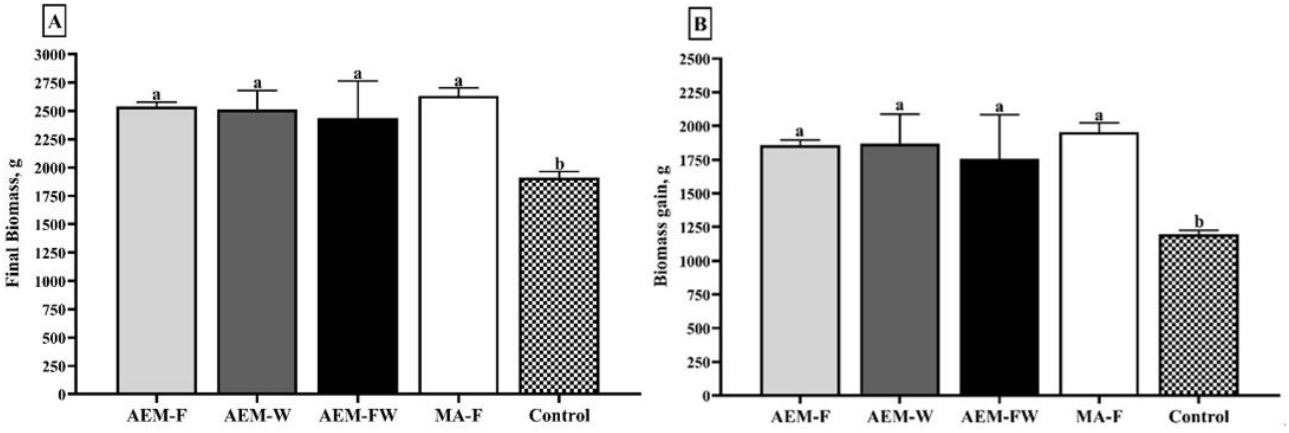

Figure 2. Final Biomass (A) and Biomass Gain (B) of shrimp fed with different strategies of AEM during eight weeks in BFT system. Values presented as mean and SD. Different minor letters represents statistical difference.

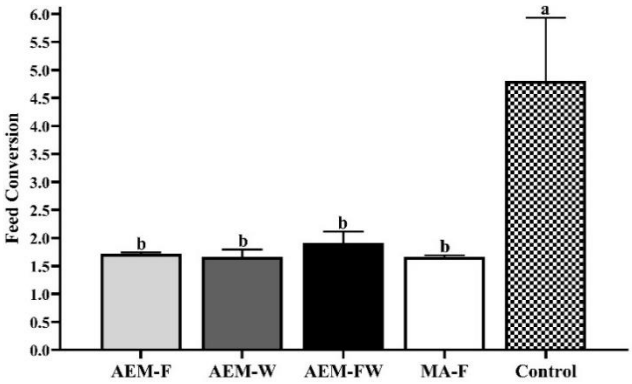

Figure 3. Feed Conversion of shrimp fed with different strategies of AEM during eight weeks in BFT system. Values presented as mean and SD. Different letters represents statistical difference.

Table 2. Zootechnical parameters of shrimp fed with different strategies of AEM during eight weeks in BFT system. Values presented as mean and pooled SEM.

	Treatments						
	AEM-F	AEM-W	AEM-FW	MA-F	Control	Pooled SEM	ANOVA p Value
[1]FW (g)	14.06	15.48	14.21	14.79	14.64	0.55	0.2809
[2]WG (g)	12.74	10.81	11.39	10.65	12.15	0.88	0.7482
[3]WWG (g)	1.33	1.51	1.35	1.42	1.52	0.08	0.0838
[4]SGR	2.53	2.71	2.55	2.62	2.71	0.08	0.0669

[1]Final Weight; [2]Weight Gain; [3]Weekly Weight Gain; [4]Specific Growth Rate (% day^{-1}).

Table 3. Water Quality parameters of shrimp fed with different strategies of AEM during eight weeks in BFT system. Values presented as mean and pooled SEM. No one-way ANOVA or repeat measures ANOVA differences were found.

	Treatments					
	AEM-F	AEM-W	AEM-FW	MA-F	Control	Pooled SEM
OD (mg L^{-1})	5.35	5.42	5.23	5.22	5.18	0.10
Temperature (ºC)	29.34	30.44	30.06	29.46	29.84	0.45
Ammonia (mg L^{-1})	0.22	0.20	0.18	0.16	0.16	0.03
Nitrite (mg NO_2^- N L^{-1})	0.20	0.21	0.20	0.18	0.19	0.01
Orthophosphate (mg PO_4^{3-} P L^{-1})	2.45	2.38	2.52	2.39	2.14	0.14
Alkalinity (mg L^{-1})	165.17	159.66	158.33	160.45	162.12	2.63
SST (mg L^{-1})	573.28	592.23	569.38	570.12	561.18	11.52
SSE (mg L^{-1})	14.08	17.66	15.22	12.38	16.02	1.99
pH	8.13	8.11	8.11	8.10	8.11	0.01

The use of AEM influenced the immunological parameters of the animals. There was no statistical difference among the different strategies of using AEM for serum protein. However, the animals that were fed with the different strategies of using AEM had a higher concentration of serum protein in the hemolymph when compared to the control treatment. Regarding the PO activity, the animals that received AEM showed higher PO activity compared to the MA-F and control treatment. The MA-F treatment showed no difference in PO activity compared to the control, and the PO activity was not influenced by the different AEM use strategies. The concentration of hemocyte (THC) was not affected using AEM. Table 4 presents the results of the immunological parameters of the animals at the end of the experiment.

Gut histomorphometry was strongly influenced by AEM use. The intestinal section area was substantially higher in the control treatment, when compared to the other treatments (Figure 4). In addition, the AEM-FW presented a lower fold area than the others supplemented treatments. Following the same effect, the length of the intestine folds (Figure 5) of the shrimp in the control group was greater than in the other treatments, followed by the MA-F, AEM-F and AEM-W (that have no difference among them), and AEM-FW, that was the lower fold length found. The width of the folds (Figure 6) was also affected, where the AEM-FW treatment had a smaller width when compared to the other treatments.

Microbiological analysis showed that the control treatment had a substantially and significantly higher concentration of the genus *Vibrio* in the intestine of the shrimp, when compared to the treatments that received AEM or MA during rearing. These results are shown in Figure 7. No statistical differences were found among treatments for heterotrophic bacteria.

Table 4. Immunological parameters of shrimp fed with different strategies of AEM during eight weeks in BFT system. Values presented as mean and pooled SEM. Different letters represents statistical difference among the results of the column.

	THC ($x10^6$ mL^{-1})	Hemolymph Protein (mg L^{-1})	PO (U mg^{-1} $min.^{-1}$)
AEM-F	7.07	2.22^a	14.13^a
AEM-W	6.96	2.20^a	16.48^a
AEM-FW	7.17	2.17^a	15.03^a
MA-F	7.16	2.17^a	11.22^{ab}
Control	7.04	2.07^b	10.16^b
Pooled SEM	0.09	0.06	2.64
ANOVA p Value	0.8784	0.0042	0.0307

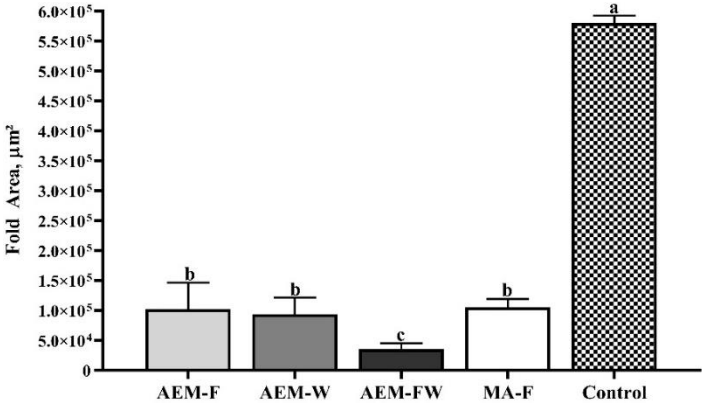

Figure 4. Fold area of shrimp fed with different strategies of AEM during eight weeks in BFT system. Values presented as mean and SD. Different letters represents statistical difference.

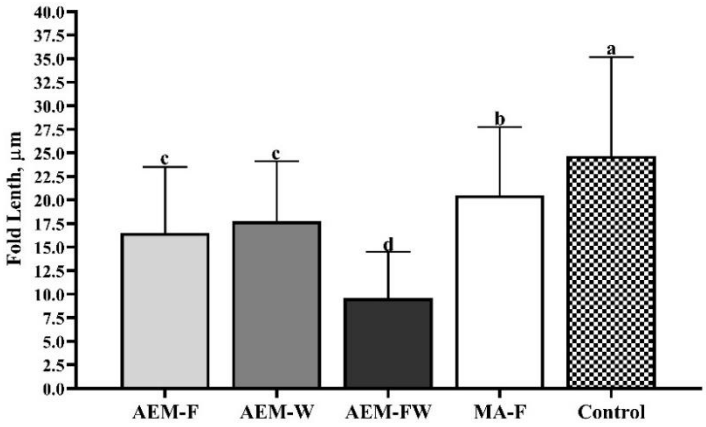

Figure 5. Fold length of shrimp fed with different strategies of AEM during eight weeks in BFT system. Values presented as mean and SD. Different letters represents statistical difference.

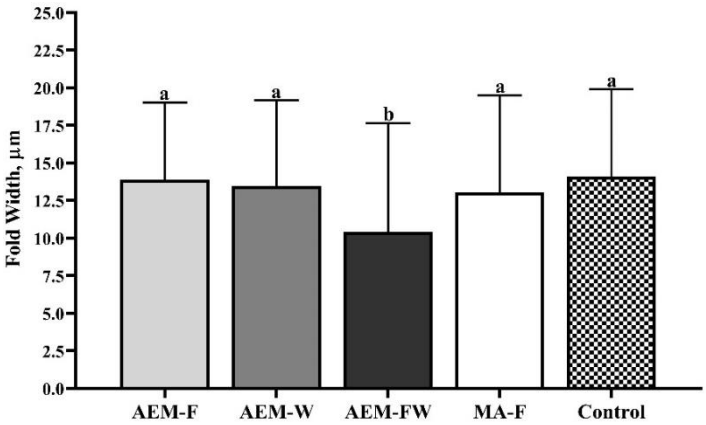

Figure 6. Fold width of shrimp fed with different strategies of AEM during eight weeks in BFT system. Values presented as mean and SD. Different letters represents statistical difference.

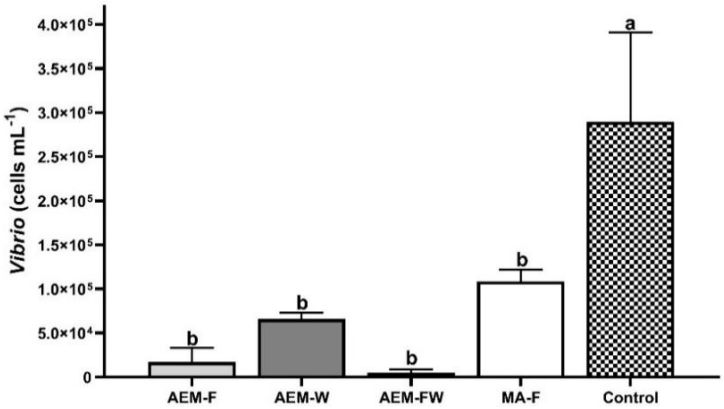

Figure 7. *Vibrio* concentration of the gut of shrimp fed with different strategies of AEM during eight weeks in BFT system. Values presented as mean and SD. Different letters represents statistical difference.

Discussion

Temperature, dissolved oxygen, pH, salinity, and nitrogenated compounds remained within the proper limits for the culture of *L. vannamei* in biofloc system (Ray, Lewis, Browdy, & Leffler, 2010; Vinatea et al., 2010; Baloi, Arantes, Schveitzer, Magnotti, & Vinatea, 2013; Pinheiro et al., 2017). The use of AEM did not affect the water quality parameters in any treatment. The inoculum used to start the BFT system was collected from a mature and stable shrimp BFT tank. In stable BFT systems, variations in water quality parameters are very small and the microbiological ecosystem maintains N and P levels within safe limits for the animals (Rajkumar et al., 2016; Jamal, Abdulrahman, Harbi, & Chithambaran, 2019). Even with the inclusion of microorganisms with bioremediation capacity, through the liquid release of AEM, the BFT system used in the experiment presented a stable and mature microbiological ecosystem, making it impossible to verify improvements in the water quality of the treatments that received the AEM in liquid form, since the system already presented stability in the water quality parameters.

The performance of *L. vannamei* was similar to that observed in other cultures with biofloc systems. Final weight and survival of the treatments that received AME or MA were close to those obtained by Ray et al. (2010) (11.6 ± 1.1 g and 71.8% respectively). Weekly weight gain was higher to that obtained by Pinheiro et al. (2017) in very similar experimental conditions, however, the feed conversion ratio of the treatments that received AEM or MA were close to that obtained by them. The control treatment showed a significantly higher feed conversion than the other treatments. This result was directly affected by survival, which was significantly lower in the control treatment. As all animals were fed based on a feeding table and the feeding was corrected weekly through weekly biometrics, the control tanks received the same amount of feed as the other treatments. However, the lowest survival was not considered in the weekly correction of feeding, since the weekly biometrics collected only one sample of animals from each tank, not allowing to determine the real size of the population of each tank. For all feed corrections it was assumed that all tanks had the same population size. This fact contributed to the higher feed conversion value found in this treatment.

The use of AEM or MA affected the histomorphometry modulation of the shrimp intestine. It is known that the composition of the diets or the presence of compounds in the media can alter the gut morphometry of the aquatic organisms. In Nile tilapia, for example, Pierri et al. (2021) verified that even micronutrients could modulate the intestinal structure of the animals. The animals in the control treatment had a fold area approximately 5 times larger than the other treatments. The same behavior, but to a lesser extent, was identified in the length of the intestinal folds of shrimp. The microbiological activator used in all other treatments is a commercial product that contains sugar, vitamins, minerals, and organic acids in its composition. The use of the MA may be the principal factor to contribute to the gut modulation of the animals from the supplemented treatments. Many compounds have the capacity to alter the gut morphometry in shrimp, such as Pineapple Waste Crude Extract (Klahan, Deevong, Wiboonsirikul, & Yuangsoi, 2023), butyrate and polyhydroxy butyrate (Silva et al., 2016), and even exposure to heavy metals, such as lead (Ding et al., 2019).

In addition to the compounds present in the MA, the way in which the microorganisms were offered to the shrimp also seems to have influenced the morphology of the intestine. All indices measured in the intestines showed lower values in the AEM-FW treatment when compared to the other treatments that received AEM or MA. This may indicate that the presence of microorganisms, when added together in the water and in the diet,

stimulated the digestive capacity of the animals, where they were able to digest and absorb nutrients from the diet more effectively, not needing to increase the intestinal absorption area. This hypothesis is corroborated by the fact that these animals did not show impairment to their growth and survival rates. This intestinal plasticity was found in many studies and similar results were found with *Clarias gariepinus* fingerlings supplemented with *Lactobacillus plantarum* (Falaye, Emikpe, & Ogundipe, 2016). In *L vannamei*, (Zhang et al., 2020) showed that the intestinal morphology of the animals varies according to the probiotic or enzymatic complexes supplemented in the feed. The author found that different additives can increase or decrease the measurements of the villi according to the microorganism offered in the shrimp diet.

Although differences in *Vibrio* intestinal count were demonstrated, no differences in total heterotrophic bacteria counts were observed among treatments in the present study. This can indicate that the presence of microorganism and/or organic compounds presented in the supplemented treatments stimulate the competition between *Vibrio* and other bacteria genus. In addition, the higher intestinal fold area increases the available surface for bacterial colonization, which increases the possibility of colonization by pathogenic bacteria as well as *Vibrio spp*. One of the main characteristics of probiotics and prebiotics is the production of compounds that can inhibit the growth of different microorganisms by decreasing the pH of their environment, increasing competition for nutrients and sites of adhesion, as well as producing antimicrobial compounds (Gatesoupe, 2007). On the other hand, in addition to decreasing pH, organic acids, or their salts, the addition of these elements can produce chelate complexes with minerals, thus inhibiting the growth of bacteria, such as those of the genus *Vibrio* (Whitaker et al., 2010). In *L. vannamei*, studies have shown that organic salts decrease the concentration of *Vibrio spp*. in intestine (Romano, Koh, & Ng, 2015; Ramírez et al., 2017).

One of the most important determinants of host immunological response in shrimp is PO activity (Ramírez et al., 2017). Melanization is an important mechanism in the immune system of shrimp as with other arthropods. By binding to specific pathogen molecules (PAMPs), the PRPs (i.e. LGBP, GBP and/or C-type lectin) detect pathogen infections and subsequently activate Clip-SPs, PPAE and proPOs to finally activate PO and generate the melanin and their reaction products (reactive oxygen compounds) that entrap and/or are toxic to pathogens (Amparyup, Charoensapsri, & Tassanakajon, 2013). The results showed that PO activity was higher in the AEM treatments when compared with all treatments. The PO enzyme may have helped to enhance host resistance to *Vibrio spp*. found in the gut of the shrimp fed with AEM or MA. Evidence has been given regarding the physiological importance of the plasma protein concentration and its susceptibility to environmental or physiological changes in shrimp species (Rodríguez & Le Moullac, 2000). Shrimp with higher plasma protein concentrations have better defense capacity against stressors or pathogens, according to results found by (Song, Yu, Lien, Huang, & Lin, 2003), which demonstrate a 56% decrease in plasma protein concentration in shrimp exposed to the Taura syndrome virus in *L. vannamei*.

The presented results demonstrate that the high mortality found in the control treatment was not related to the water quality parameters. However, the other markers used in this study suggest that the presence of AEM or MA stimulated the immune system of the animals, in addition to contributing to the lower concentration of *Vibrio* through microbial competition or production of antimicrobial compounds. As a modulation for better digestion and nutrient absorption, the increase in the intestinal area and villi may have contributed to the increase in *Vibrio* concentrations in the intestines of the animals, since the area of intestinal colonization was greater in the control treatment. Thus, the increased area of villi associated with lower immunological capacity and lower microbial competition appears to have contributed significantly to the higher concentration of *Vibrio* in animals in the control treatment. The presence of these potentially pathogenic microorganisms appears to be the cause for the high mortality of these animals.

Conclusion

The use of AEM or MA, although it did not affect the growth, stimulated the digestive process of the animals, allowing a greater absorption of nutrients with a smaller intestinal area. Associated with this, the presence of additives stimulated the immunological parameters of the animals and reduced the presence of *Vibrio* in the intestine of the shrimp. The association of these factors meant that the survival of the animals that received the additives in the diet was substantially higher than that of the animals that did not receive the additives. Additionally, it was verified that the BSE supplementation strategies did not differ among themselves, and the producer could opt for the strategy of lower cost and/or less complexity of use, according to each specific case.

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