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ISSN 0792 - 156X

PUBLISHER:

The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB)

The Israeli Journal of Aquaculture - Bamidgeh, IJA_70.2018.1533, 13 pages

Published as an open-access journal by the Society of Israeli Aquaculture & Marine Biotechnology (SIAMB).
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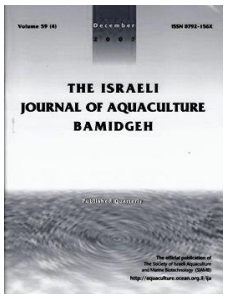

Padina caulescens and *Schizochytrium* sp. as Supplemented Feed in the Nursery Production of *Penaeus vannamei* Post Larvae Reared in Biofloc Systems

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Keywords: Biofloc; macroalgae; microalgae; shrimp; probiotic

Abstract

The appearance of diverse diseases in cultured shrimp has led to the search for alternative prevention strategies. One of these alternatives is Biofloc technology (BFT). However, this requires further optimization. The objective of this work is to improve this system by adding microalgae (*Schizochytrium* sp.), probiotic bacteria (*Lactobacillus fermentum*), and macroalga (*Padina caulescens*), which in addition to maintaining the quality of water in the *Penaeus vannamei* culture, improves growth parameters at the maternity stage. We installed an experiment that included six different treatments in culture tanks containing 250 L of seawater each, and 500 shrimp (68 mg) m/3, and evaluated the results for 35 days, in which we gradually decreased the inert-feed to 50-75% and substituted it with 50-25% of *P. caulescens*.

The results showed that, in comparison to the control, survival, and final weight of the shrimps improved and improved further with the use of probiotic bacteria, *Schizochytrium* sp. and *P. caulescens* and probiotic bacteria enabled a reduction of 50% of the inert feed. Therefore, we recommended the use of *P. caulescens, Schizochytrium* sp. (LPU-1), and *L. fermentum* (T-19) in BFT cultures for *P. vannamei*.

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Introduction

Of the different species of shrimp cultivated in Latin America, the white shrimp (*Penaeus vannamei*) is more frequently cultivated and with increasingly technological systems. Biofloc technology systems (BFT) have comprised one of the most viable options for high-density culture conditions, limiting the exchange of water and the accumulation of nitrogenerated compounds and outbreaks of disease in shrimp. The current development of BFT is towards to recycling nutrients, aeration systems, and diets adequate for support the nutrient generated by microbiota in the systems (Su-Kyoung et al., 2014). In this regard, these systems are increasingly proposed for the production of microbial communities that, in addition to the reduction and minimizing water exchange. They have the additional advantage of promoting microbiota with high protein value that is consumed directly or that can be supplied in inert feed enhancing growth immunity (Panantharayil Sudhayam et al., 2017). These biofloccules provide an alimentary support of 37-40% juvenile shrimps in BFT systems and growth of *Litopenaeus stylirostris* and *Litopenaeus vannamei* (Cardona et al., 2015; Dantas et al., 2016).

The use of microbiota as bioregulators in water quality and nutrimental support in BFT systems has been proven. The use of marine microalgae in other types of systems (e.g., open systems, integrated multitrophic cultures) is mainly used to maintain the quality of the water (i.e., Otavio Brito el at., 2014; Samocha et al., 2015; Baloo et al., 2014), as partial feed in semi-intensive cultures of shrimp (Pallaoro et al., 2016), and as an ingredient in inert feeds for shrimp in culture (Elizondo-González et al., 2017; Gamboa-Delgado et al., 2011). Microalgae, and macroalgae, in addition to their high protein content, include also β-carotene; astaxanthin; docosahexanoicacid; eicosahexanoicacid; phycobilinpigments; polysaccharides, and amine essential acids (Borowitzka, 2013; Wells et al., 2017). The latter can be lost at times during the production process but could be employed within the BTF system combined with macroalgae. In this respect, the microalga *Schizochytrium* sp. has been evaluated as an inert feed additive for *P. vannamei*, finding that, despite their low protein content, have good zootechnical results with *P. vannamei* (Pacheco-Vega et al., 2018).

In the feeding processes of marine crustaceans under culture, more than 50% of their costs are represented by the inert feed (Martínez-Córdova et al., 2017), recent studies show the benefits of supply of natural food in the cultivation of shrimp (i.e., Dantas et al., 2016; Gamboa-Delgado, 2014; Cardona et al., 2015). Additionally, the authors show that, the inert food can be replaced by means of live food, diminishing the need of formulated feed for the shrimp culture. In this work, we proposed to optimize feed in hyper-intensive cultivation of white shrimp *P. vannamei* maintained in a BTF system induced by the supply of micro-aggregated (microalgae plus probiotic bacteria) and endemic macroaggregates (macroalgae) for the diminution of *P. vannamei* inert feed.

Materials and Methods

Bioassay with shrimp

The experimental layout that was installed outside of the Laboratory contained 18 translucent fiber glass tanks with a capacity of 300 L each that were covered individually and installed in wooden structures that support five tanks each. The operable level for each tank was 250 L filled with filtered (1 μm) seawater and disinfected with a solution of hypochlorite at a ratio of 1mL chlorine /1L water for 24 hours and inhibited with thiosulfate sodium at 0.05g/1mL. The salinity was maintained at 30 ppt. For each treatment, three experimental units were utilized (six treatments and three repetitions) that were provided with aeration through a micropore hose.

The BFT maturation process consisted of an initial seeding with *Schizochytrium* sp. (microalga) and *Lactobacillus fermentum* (probiotic bacteria); F/2 medium (unique dose) as a nutrient source and sugarcane molasses as a carbon source at a concentration of 9 g/m^3 daily for 6 days. Prior to the seeding of shrimp (maturation period), the microalgae and the probiotic bacteria were seeded in the tanks, at an initial density of 100 899 cells/mL y $4.8x10^3$ Colony-Forming Units (CFU)/ 1mL, respectively.

The macroalgae supplied to the BFT cultures were based on the desired percentage (36%) of protein, substituting partially the protein of the inert feed for that of the microalgae (Table 1). The experiment started with a daily feeding ratio of 20% of the wet shrimp weight, distributed in three feeding rations. Before the beginning of the experiment, we carried out a bromatological analysis (i.e proteins, lipids and carbohydrates) of the microalgae that provided information about its nutritional value and facilitating the determination of the amount of macroalgae needed for the substitution of the protein of the inert feed (Malta Cleyton, Api Shrimp, with 40% protein and 3.5 fiber).

Table 1. Experimental conditions and feeding ratio evaluated, followed by 5 weeks under culture of *P. vannamei* maintained in a BFT system

Treatment	*Culture condition*	*Rate-fed pellets: macroalga (%:%) % of protein*
T1	Microalga + probiotic	100:0 (control)
T2	Only probiotic bacteria	100:0
T3	Microalga + probiotic bacteria	75:25
T4	Microalga + probiotic bacteria	50:50
T5	Microalga + probiotic + Macroalga	75:25
T6	Microalga + probiotic + Macroalga	50:50

Microalga: *Schizochytrium* sp.; probiotic: *Lactobacillus fermentum*; Macroalga: *Padina caulescens.*

The feeding started with one session of 20% per week and following the feeding routines of table 3.

Seeding of post larvae

The post larvae of *P. vannamei* (PL15) were provided by the commercial laboratory: Laboratorio Acuícola Alápsa. The shrimp post larvae were acclimatized to lower salinity, from 34 to 30 ppt during 2 weeks, up to 68 mg ± 20 mg (individual average). The density of the seeding was 500 org/m^3 (125 PL per unit). Additionally, we took samples (15 mL) of the water of each culture unit to assess the microalga (*Schizochytrium* sp.) counts, as well as those of the probiotic bacteria (*Lactobacillus fermentum*) and samples of 50 mL for bromatological analysis, that include proteins, lipids, and carbohydrates.

Origin, culture, and scaling of algae and probiotic bacteria

The experimental development and analysis of the constituents of the macroalgae and of BFT systems described here was carried out in the Production Laboratory of Live and Inert Feed of the National School of Fishing Engineering (ENIP) of the Autonomous University of Nayarit, localized in the Bay of Matanchén, Nayarit, Mexico on the Pacific coast.

The microalgal strain of *Schizochytrium* sp. belongs ENIP's strain collection of live feed. These strains are maintained in F/2 medium (Guillard, 1975) in 10 mL assay tubes at a temperature of 23 °C, under constant illumination with daylight incandescent lamps. The probiotic bacteria strain *Lactobacillus plantarum* belongs to the Laboratory collection, which was isolated from the digestive tract of the white shrimp (*P. vannamei*) and maintained in MRS medium (Millipore). *P. caulescens* macroalgae were collected along the coast of the Matachén Bay, Nayarit, maintained in seawater filtered at 1 µm under continuous aeration, and in F/2 medium as nutrient source.

The microalgae were up-scaled through a series of progressively larger Erlenmeyer flasks (500 mL), inoculated into filtered seawater, and sterilized with ultraviolet light and in autoclave (24 LT ALL AMERICAN) at 121°C for 20 min at pressure of 1.02 kg/cm^2. Larger volume cultures (400 L) were grown in fiberglass tanks and the water used was filtered and cleaned utilizing 1 mL of hypochlorite per liter of seawater to inhibit the action of the chlorine. We used sodium thiosulfate at a ratio of 0.05 g/1 mL of hypochlorite. Incubation was carried out at 23°C, and there was

constant illumination with daylight incandescent lamps for flasks and water jars, with the exception of the 400-L cylinder, where the culture was grown outdoors without controlled temperature and with natural day:night photoperiod.

The probiotic bacterium were obtained from the strain maintained in MRS-Agar medium. It was scaled in an assay tube with MRS broth for its scaling up to the 17L polyethylene carboy. The water (seawater) was cleaned according to the method previously mentioned for sodium hypochlorite and incubated at 30°C for 48 hours at each stage. For cultures of 17L with culture shrimp we used sugarcane molasses as the carbon source for bacteria.

The macroalga *P. caulescens* was selected due to its endemic abundance and easy collection from the inter sea zone, which is naturally detached from the marine benthos due to action of the waves and currents. This in turn expels the macroalga to the shoreline of the coast, representing a potentially advantageous resource that does not impact on the ecology. The macroalga was transported to the laboratory and maintained in seawater enriched with F/2 medium, with constant aeration, without temperature control, and with day:night photoperiod. Before feeding, the macroalgae were submitted to an osmotic-shock process by immersion in fresh water for 20 minutes and triturated into squares (0.5 x 0.5 cm^{-2}) to improve microorganism colonization and serve as feed for the post-larval shrimp according to the defined feeding procedure.

Bromatological and chlorophyll analyses

The bromatological analysis of the floccules was performed at the beginning, middle, and end of the experiment. The floccules (day 0) were collected by centrifugation at 3,200 rpm x 30 min of 90 mL of water per tank. The remaining biomass was then washed with distilled water for 10 minutes under the same conditions. The second sampling at day 17, was conducted using 11 filterings of the culture with a 30-micra light sieve. These were also washed with distilled water. Changes in the composition of the macroalgae *P. caulescens* were determined before being added to the cultures, and once incorporated at day 0, 17 and 35. The floccules and the macroalgae were dehydrated at 60°C for 48 hours and stored at 23°C until being analyzed. Protein content was obtained according to Lowry et al. (1951) and modified by Malara and Charra (1972). Carbohydrate content was determined according to White (1987) and Dubois et al. (1956). Lipids were determined according to Bligh and Dyer (1959) with the modification of Chiaverini (1972).

The assessment of the cellular density versus the concentration of chlorophylls in *Schizochytrium* sp., in a sample of 18 mL of monoalgal culture, was performed by direct recount of the sample with a hematocytometer slide (0.1 mm deep), and determined chlorophylls a, b, and c following the methodology described by Jeffrey and Humphrey (1975) with a spectrophotometer (ThermoScientific, Genesys 10S Vis). At the beginning, middle, and end of the experiment, we took samples from all the cultures and froze them under dark conditions at -17°C to determine the concentration of microalgae and chlorophylls.

Water quality

Variables of water quality in cultures were recorded three times daily (8 am, 1 pm, 6 pm). These included temperature (°C), dissolved oxygen (mg/L), pH, and salinity determined using a refractometer (ppt) (RHS-10 ATC). Determination of ammonium (NH_4^+), nitrites (NO_2), nitrates (NO_3), phosphates (PO_4), and carbonates (Na_2CO_3) in the cultures was performed every 7 days, following the spectrophotometric procedures described in the LYSA commercial kits, based on the principle of Strickland and Parsons (1972). Water exchange was carried out at a rate of 5% every 2 days, and salinity was kept constant at 30 ppt. The seawater and fresh water utilized for the exchanges were submitted to a salinization process as follows: filtration at 5 and 1 µm; sodium hypochlorite at 50 ppm for 24 h; and neutralization with sodium thiosulfate. As a carbon source for probiotic bacteria, we employed sugarcane molasses sterilized by autoclaving at 15 lb PSI for 20 min, adding daily the amount determined according to the methodology described by Avnimelech (1999).

Microbiological analysis

We carried out a follow-up of the concentration of total lactic bacteria (including probiotic) every 7 days in the cultures by means of 100-µL seedings on Petri dishes with MRS-agar medium and incubated at 30°C ±1°C for 24 hours and with direct counts of CFU per mL (UFC /mL). At the end of the experiment, we took two shrimps at random from each experimental unit to evaluate the concentration of lactic acid bacteria and bacteria of the genus *Vibrio* spp. present in shrimp tissue. The samples received a bath of 70% alcohol and sterile distilled water; they were macerated with 1 mL of sterile seawater, centrifuged at 3,200 rpm x 15 min, and we took 100-µL for seedings on Petri dishes with RS-agar and TCBS-agar.

Shrimp performance

At the end of the 5 weeks of experimental culture, we determined the following parameters: weight gained (g); final biomass (g); survival (%), and the feed conversion rate (FCR) according to Zhao et al., (2012). These parameters were obtained with the initial data, feeding, and total harvest of the organisms at the end of the experiment.

Statistical analysis

Prior to analysis, the data were tested for homoscedasticity and normality (Bartlett and Kolmogorov-Smirnov tests). The results obtained for final mean of the chlorophyll (*a*, *b*, and *c*), weight, survival rate, weight gain, and the FCR were subjected to an analysis by one-way Analysis of Variance (ANOVA) ($P > 0.05$). Total protein, total carbohydrates, total lipids, and ash percentages were transformed using arcsine prior to statistical analysis and analyzed by two-way ANOVA ($P > 0.05$) and Tukey post-hoc analysis when needed. Statistical analyses were performed using Statistical ver. 8 software for Windows.

Results

Water quality parameters

Water quality is essential for the correct functioning of the cultures. The results of water quality showed adequate conditions for the culture of *P. vannamei*. The concentration of dissolved water, the temperature, and the salinity were maintained within ranges similar amongst the treatments. The concentration of chlorophyll in cultures varied among treatments, the latter including the following: microalga + probiotic, and microalgae + probiotic + macroalgae/75, those of greatest concentration (Table 2). Of the different chlorophylls, the chlorophyll presented in the different cultures at different proportions; however, chlorophyll *a* and *b* presented in trace amounts in the treatment with the probiotic.

Table 2. Effect of BTF and its different constituents in the different water-quality parameters of *P. vannamei* culture fed with different rates of inert feed for 35 days.

	Parameters (min-max)				*Chlorophyll* (µg/L)*		
Treatment/Feed pellets rate (%)	*Dissolve oxygen (mg/L)*	*Temperature (C)*	*Salinity (ppt)*	*pH*	*a*	*b*	*c*
T1	5.0-6.2	21.7-26.6	28.3-30.6	8.0-8.4	15.2-256.0	2.0-12.8	0.1 – 45.3
T2	4.8-5.6	21.6-27.1	29.0-30.0	8.0-8.3	12.6 – 45.5	11.5 – 0.6	0.3 -4.8
T3	5.1-6.0	20.6-27.7	29.8-30.3	8.1-8.4	28.8 -61.6	9.4- 5.3	8.2 10.0
T4	4.7-5.9	21.0-26.3	28.0-30.0	8.1-8.4	140.1- 128.8	29.9 -18.6	21.2 – 22.3
T5	4.9 -6.3	21.4-28.5	29.3-31.0	7.5-8.2	281.1 – 295.0	45.7 -30.6	44.4 -43.2
T6	5.1– 6.4	21.1-27.0	28.6-31.3	7.7-8.4	120.9 – 102.4	22.2 -16.4	23.4 – 18.5

*Average values at days 17 and 35 of culture.

T1 = *Schizochytrium* sp. + *L. plantarum* /100; T2 = *L. plantarum* /100; T3 = *Schizochytrium* sp. + *L. plantarum* /75; T4 = *Schizochytrium* sp. + *L. plantarum* /50; T5 = *Schizochytrium* sp. + *L. plantarum* + *P. caulescens* /75, and T6 = *Schizochytrium* sp. + *L. plantarum* + *P. caulescens* /50.

The changes in the nitrogen compounds, including ammonium, nitrites, and nitrates, are shown in Figure 1. They show that from day 17 of culture the nitrogen compounds presented a variation due to number of days of culture, and not due to the treatment, presenting an increase in ammonium of 0-2.8 mg/L, and a concentration of the nitrites between 0-0.93 mg/L, and nitrates between 0-10.4 mg/L. The concentrations of phosphates and alkalinity are depicted in Figure 2: with concentrations of 0.09-1.3 mg/L of phosphates and 18-280 mg/L in alkalinity.

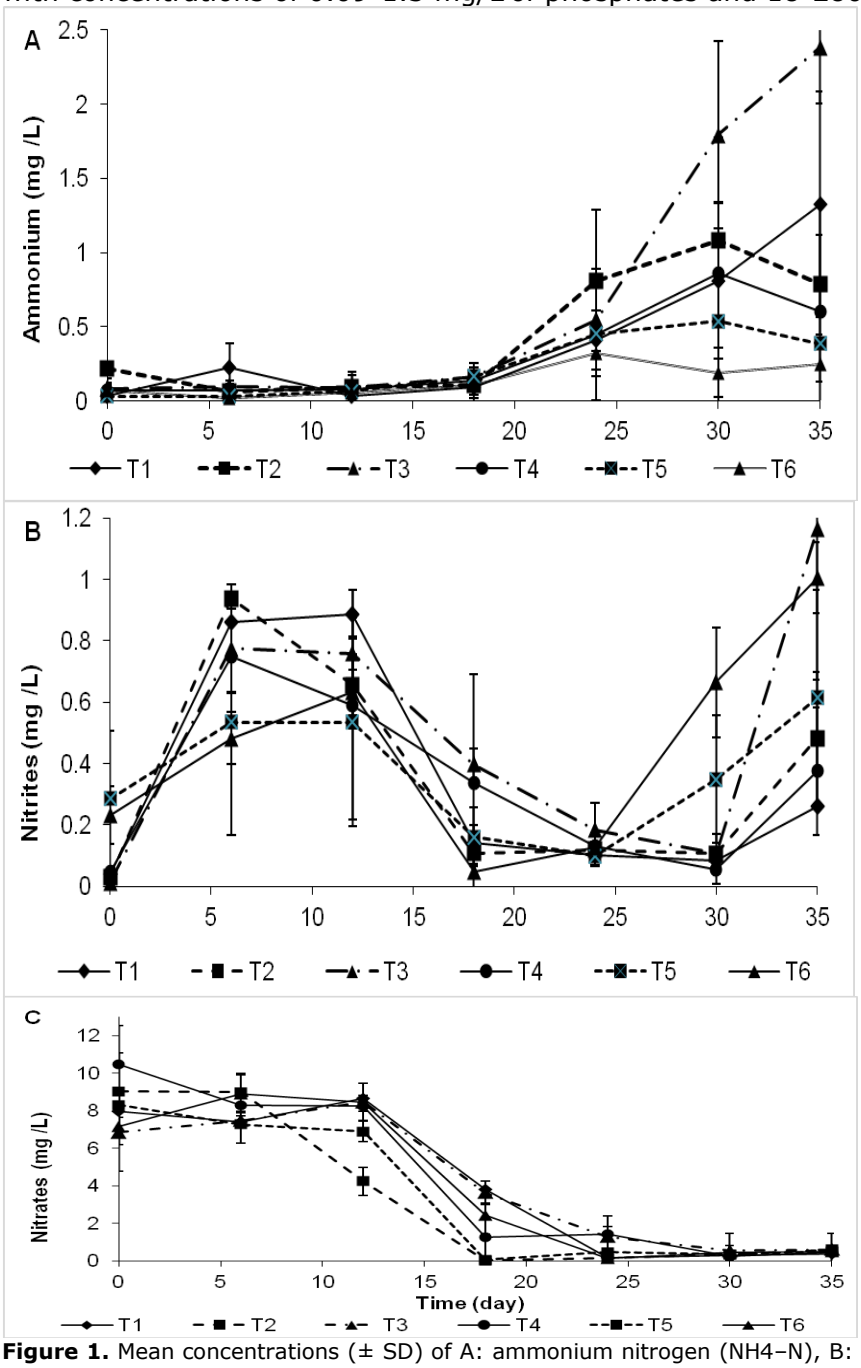

Figure 1. Mean concentrations (± SD) of A: ammonium nitrogen (NH4–N), B: nitrite nitrogen (NO2–N), and C: nitrate nitrogen (NO_3–N) in the culture *of P. vannamei* maintained in BTF under different culture conditions. The inert feed rate and inert feed rate in the BTF system, where: T1 = *Schizochytrium* sp. + *L. plantarum* /100; T2 = *L. plantarum* /100; T3 = *Schizochytrium* sp. + *L. plantarum* /75; T4 = *Schizochytrium* sp. + *L. plantarum*_/50; T5 = *Schizochytrium* sp. + *L. plantarum* + *P. caulescens* /75, and T6 = *Schizochytrium*_sp. + *L. plantarum* + *P. caulescens* /50. The vertical bars identify the variation of the means.

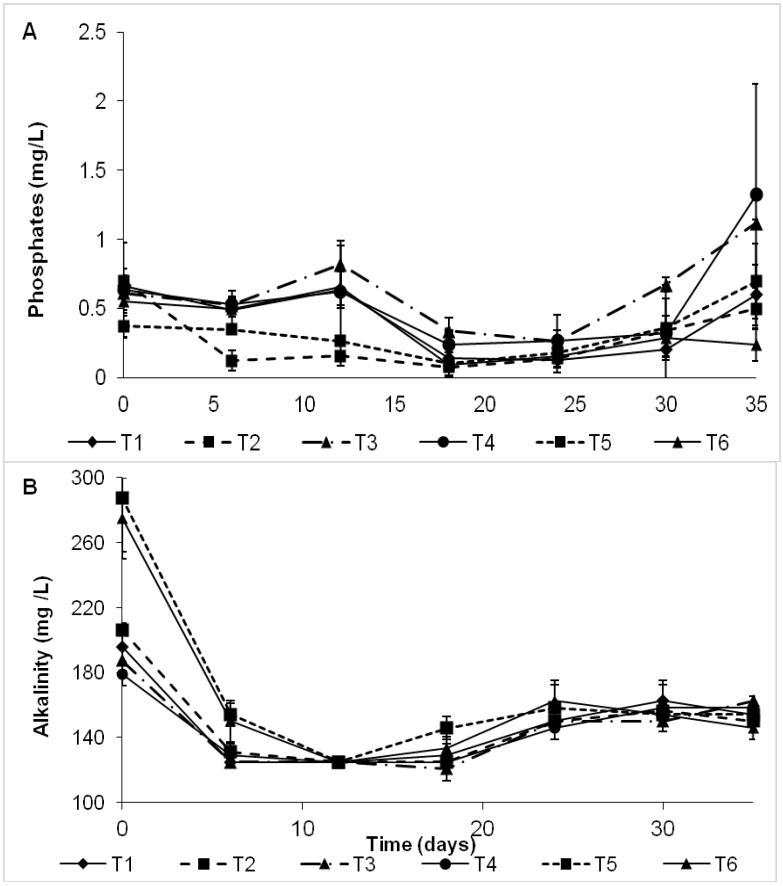

Figure 2. Mean concentrations (± SD) of A: phosphates (PO^{3-} 4) and C: alkalinity (CaCO3) in the culture of *P. vannamei* maintained in BTF under different culture conditions. The inert feed rate and inert feed rate in the BTF system, where: T1 = *Schizochytrium* sp. + *L. plantarum*_/100; T2 = *L. plantarum* /100; T3 = *Schizochytrium*_sp. +_*L. plantarum* /75; T4 = *Schizochytrium* sp. + *L. plantarum* /50; T5 = *Schizochytrium* sp. + *L. plantarum* + *P. caulescens* /75, and T6 = *Schizochytrium* sp. + *L. plantarum* + *P. caulescens* /50. The vertical bars identify the variation of the means.

The concentration of lactic acid bacteria (LAB) in the cultures diminished from 1,000 CFU/mL on average on day 0, to 100 CFU/mL on day 15 of culture. After that date, the concentrations of total LAB were maintained constant (Figure 3). Also, in shrimp tissue, the concentrations showed differences among treatments, with a concentration of 166 CFU/g of tissue in the treatment with microalga + probiotic + macroalgae, and the least concentration in the treatments: probiotic, microalgae + probiotic, and microalgae + probiotic + macroalgae (Figure 4).

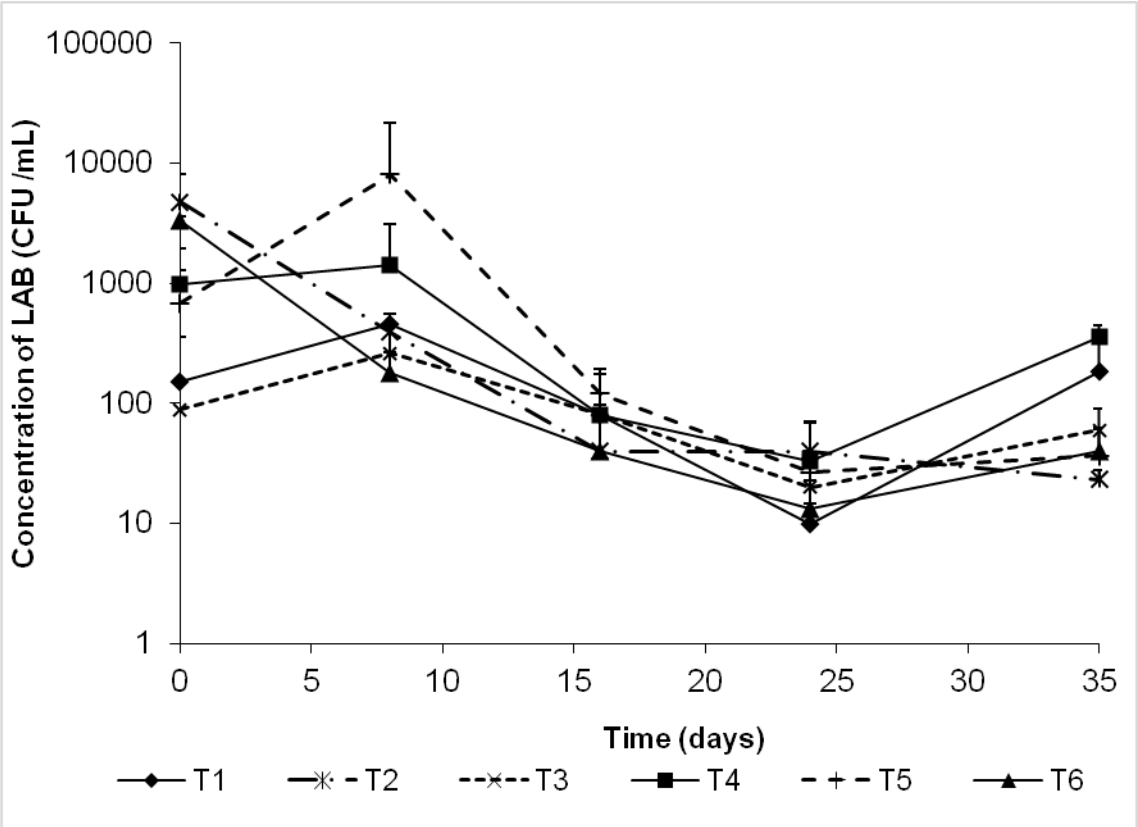

Figure 3. Concentration of lactic acid bacteria (LAB) (CFU /mL) in the water of cultures of *P. vannamei* maintained in BTF under different culture conditions. The inert feed rate and inert feed rate in the BTF system, where: T1 = *Schizochytrium* sp. + *L. plantarum* /100; T2 = *L. plantarum* /100; T3 = *Schizochytrium* sp. + *L. plantarum* /75; T4 = *Schizochytrium* sp. + *L. plantarum* /50; T5 = *Schizochytrium* sp. + *L. plantarum* + *P. caulescens* /75, and T6 = *Schizochytrium* sp. + *L. plantarum* + *P. caulescens* /50. The vertical bars identify the variation of the means.

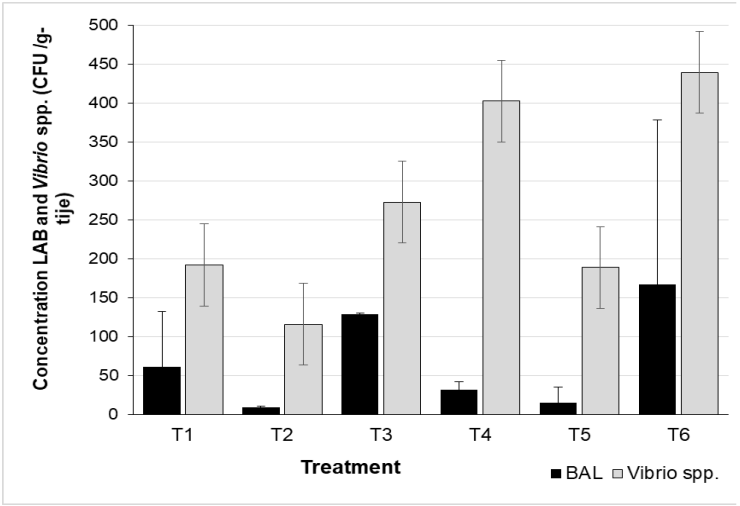

Figure 4. Concentration of lactic acid bacteria (LAB) and *Vibrio* spp. (x10^3) in shrimp tissue on day 35 of culture of *P. vannamei* maintained in BTF culture under different culture conditions. The inert-feed rate and the inert feeding rates in the BTF system, where: T1 = *Schizochytrium* sp. + *L. plantarum* /100; T2 = *L. plantarum* /100; T3 = *Schizochytrium* sp. + *L. plantarum*____/75; T4 = *Schizochytrium* sp. + *L. plantarum* /50; T5 = *Schizochytrium* sp. + *L. plantarum* + *P. caulescens* /75, and T6 = *Schizochytrium* sp. + *L. plantarum* + *P. caulescens* /50. The vertical bars identify the variation of the means.

Bromatological analysis

The bromatological composition of the BTF system on the three different sampling dates of the culture, including initiation, middle, and end of the experiment, demonstrated that the protein content of the BTF system increased gradually with the passage of time. In contrast to the content of total protein, the lipid content in the BTF system exhibited an inverse tendency to that shown in the concentration of proteins; that is, the content of lipids diminished at dates after day 0 of culture. The content of total carbohydrates did not vary in this research (Table 3).

Table 3. Bromatological composition of BTF induced and macroalgae at different culture dates of *P. vannamei* maintained in BFT and fed with different rates of inert feed.

Treatment	*Feed pellet rate (%)*	*Days of culture*	*Protein (%)*	*Lipids (%)*	*Carbohydrates (%)*
T1	100	0	21.8 ± 2.3	41.8 ± 9.9	4.1 ± 0.2
		17	31.7 ± 0.0	32.1 ± 2.2	5.2 ± 0.9
		34	42.0 ± 5.4	27.1 ± 2.3	5.0 ± 1.1
T2	100	0	10.5 ± 1.8	22.2 ± 0.8	5.0 ± 0.9
		17	34.5 ± 4.6	20.0 ± 2.3	5.3 ± 0.8
		34	38.7 ± 2.5	26.6 ± 0.2	6.5 ± 0.5
T3	75	0	17.9 ± 0.7	35.5 ± 1.6	2.2 ± 0.1
		17	33.8 ± 1.8	36.1 ± 1.7	4.0 ± 1.14
		34	28.3 ± 1.8	22.7 ± 3.5	3.6 ± 0.5
T4	50	0	27.3 ± 2.8	30.9 ± 5.5	5.6 ± 0.6
		17	24.8 ± 0.1	27.3 ± 0.4	5.2 ± 0.2
		34	29.3 ± 3.7	25.2 ± 3.7	6.1 ± 0.5
T5	75	0	29.2 ± 2.5	37.0 ± 3.7	6.7 ± 0.4
		17	29.7 ± 2.0	23.4 ± 0.4	4.9 ± 0.6
		34	37.1 ± 2.0	24.2 ± 5.1	5.6 ± 1.7
T6	50	0	23.3 ± 1.2	25.6 ± 3.9	4.1 ± 0.1
		17	33.7 ± 4.1	30.5 ± 4.7	3.6 ± 1.1
		34	45.8 ± 1.2	22.2 ± 2.2	4.1 ± 1.9
Macroalga: *Padina caulescens*	Clean BTF	0	36 ± 5.3	21.5 ± 7.2	5.6 ± 0.04
	With BTF	17	45.3 ± 2.8	20.7 ± 2.6	7.8 ± 0.3

T1 = *Schizochytrium* sp. + *L. plantarum* /100; T2 = *L. plantarum* /100; T3 = *Schizochytrium* sp. + *L. plantarum* /75; T4 = *Schizochytrium* sp. + *L. plantarum* /50; T5 = *Schizochytrium* sp. + *L. plantarum* + *P. caulescens* /75, and T6 = *Schizochytrium* sp. + *L. plantarum* + *P. caulescens* /50.

The performance obtained in *P. vannamei* showed that the diminution of inert feed in the BFT system induced with probiotic bacteria did not exert a significant effect on the indicators of performance. However, the inclusion of *P. caulescens* macroalgae to the cultures did indeed have a beneficial effect, improving survival and FCR (Table 4). We observed that the diminution of inert feed and substitution of microalgae based on the protein content of both, favors survival and FCR in *P. vannamei* maintained in the induced BFT. In addition, the use of probiotic bacteria herein evaluated regarding the BFT, results in being the least favorable culture condition, and a diminution in inert feed implies its substitution by another source, with the macroalga *P. caulescens* an option for improving the performance of BFT in ambits similar to the conditions evaluated herein.

Table 4. Average values ± standard deviation (SD) of performance indicators in SD with *P. vannamei* under different culture conditions as follows: diminution of the inert feed rate and the inert feed rate in BTF systems a > b > c

Treatment	Feed pellet rate (%)	Final weight (g)	Survival (%)	Feed conversion ratio
T1	100	1.60 ± 0.01	63.6 ± 7.3	1.25 ± 0.14^{ab}
T2	100	1.33 ± 0.06	66.4 ± 0.0	1.31 ± 0.00^{ab}
T3	75	1.34 ± 0.52	79.2 ± 14.5	0.98 ± 0.37^{ab}
T4	50	1.45 ± 0.27	67.4 ± 9.3	0.95 ± 0.08^{ab}
T5	75	1.60 ± 0.73	81.6 ± 6.7	0.98 ± 0.37^{ab}
T6	50	1.37 ± 0.05	76.5 ± 15.8	0.62 ± 0.02^{b}

T1 = *Schizochytrium* sp. + *L. plantarum* /100; T2 = *L. plantarum* /100; T3 = *Schizochytrium* sp. + *L. plantarum* /75; T4 = *Schizochytrium* sp. + *L. plantarum* /50; T5 = *Schizochytrium* sp. + *L. plantarum* + *P. caulescens* /75, and T6 = *Schizochytrium* sp. + *L. plantarum* + *P. plantarum* /50.

Discussion

In this study, the environmental parameters of oxygen, temperature, salinity, and pH in the water of different experimental treatments did not present variations among themselves and were similar to the concentrations suggested for the culture of *P. vannamei* in BFT (Mendoza-López et al., 2017). The BFT system evaluated here was induced by *Lactobacillus fermentum* (T-19), LAB, and the microalgae *Schizochytrium* sp. (LPU-1). The presence of the microalga seeded at the onset of the experiment reflects the concentration of chlorophyll that is characteristic of this genus *a* and *b* in culture, with the latter's main photosynthetic constituents, as shown in these results. These are characteristic of this genus (*Schizochytrium* sp.*)* and of green algae (Rajkumar et al., 2013). The treatment with probiotic only resulted in the lowest concentration of chlorophyll (*a* and *b*), which can be attributed to the presence of photosynthetic bacteria (Bryant et al., 2012) that develop in the culture systems; these chlorophylls increased in treatments with microalgae and macroalgae. This increase is qualitative, and the presence of chlorophyll *c* revealed that, besides *Schizochytrium* sp., other species of algae and bacteria entered the system. This species input might have occurred due to water exchange and adherence of epiphytes to the macroalgae *P. caulescens*. The high concentration of algae and bacteria suggests that their interaction maintains the optimal concentrations of nitrogenous compounds such as ammonium, nitrites, and nitrates in this type of culture (Emerenciano et al. 2017).

The concentration of total LAB in cultures did not present important changes among treatments through time, in contrast with the methodologies for BFT, where LAB concentration is 10^7-10^8 CFU/mL (Burford et al., 2003). Nonetheless, these bacterial concentrations in the cultures were sufficient for the fixation and transformation of nitrogenous compounds and phosphates generated in the system. Given that, in addition to compiling the characteristic aspects of probiotics, described by Sivakumar et al. (2014), probiotic bacteria (T-19) are native to the shrimp-culture areas, thus conferring high adaptation to the culture conditions and presenting high performance on the removal of these compounds. Their presence also explains the diminution of Vibrio in the probiotic experiment competition present in these bacteria versus probiotic bacteria for food being displaced by the former.

In this study, we sought to induce BTF with bio-aggregates (bacteria, microalgae, and macroalgae) native to tropical environments so that they would be maintained in the systems along the culture without re-seedings of microalgae and probiotic bacteria. In the results presented here, a variation was obtained in the composition of the floccules due to the effect of the culture time and according to the bio-aggregates evaluated. In the former case, this is due to the entry of diverse bacterial flora, phytoplankton, rotifers, ciliates and flagellates, protozoa, and copepods, which tend to enter the cultures, evident from the increase of organic matter (proteins, lipids, and carbohydrates) in the macroalgae *P. caulescens.* With regard to the bromatological changes caused by the different bio-aggregates, a lower protein content was obtained when using only probiotic bacteria (control) in the cultures. This is evidenced by changes in the composition of the macroalgae *P. caulescens* prior to inclusion into the BFT system and after 17 days, when colonized by fouling (bacteria, probiotic bacteria, microalgae, and zooplankton), where a protein increase of 8.8% was observed. These changes were reported by Porchas-Cornejo et al. (2013) mainly for copepods, which comprise up to 83% of the zooplankton.

There was no defined tendency seen in the composition of lipids in the BTF system, as in the case of proteins. In the latter, we observed a lower lipid content in the treatment with probiotics only, probably because the lipid content in bacteria ranges between 1%-3% (Gamboa-Delgado and Márquez-Reyes, 2016). Therefore the low bacterial concentration (< 10 x10^3 CFU/mL); these lipids are not reflected in the lipid concentration in the microbial floccules of cultures. Rather they are represented in algae, protozoa, rotifers, and cyanobacteria that improve the nutritional quality of the BTF system (Emerenciano et al., 2012), and demonstrates the importance of lipid contribution to algae in feed consumed by *P. vannamei*.

The protein in the floccules, increased from day 0 to day 35, which correlates with the concentration of concentric diatoms in the BFT, system that tend to develop progressively in the cultures (Fernández Da Silva et al., 2008). The probiotic supplement in itself does not offer an important source of available protein; together with algae it further enhances protein by adding them as an additional substrate. This finding is in agreement with our results where we used microalgae and macroalgae as BFT promoters, obtaining a greater protein content.

The performance results obtained here showed improvement in shrimp by adding macroalgae to the cultures. The diminution of inert feed and the substitution of macroalgae in an environment with *Schizochytrium* sp., probiotic bacteria *Lactobacillus* spp., and the macroalga *P. caulescens* further improved their economical performance. Under these culture conditions, the administration of inert feed can be reduced by up to 50%, significantly reducing FCR. This result corresponds to results from analysis of stomach content in *P. vannamei* cultivated in tanks, where 51% of natural food was registered in the shrimp intestine, and the remainder was inert feed (Gamboa-Delgado, 2014).

Conclusion

The results obtained herein show that a BFT system with microalgae and macroalgae improved the efficiency in *P. vannamei* during the first fattening stages. With these results, an FCR of 0.62 ± 0.02 can be obtained with survival similar to the control. This indicates that *P. vannamei* very efficiently utilize microbiota generated in the BFT system, and this also saves expenses, and reduces environmental pollution.

Acknowledgments

The authors wish to thank the Project: Apoyo a la Incorporación de Nuevos PTC-PRODEP. Clave interna SIP16-190, CONACYT PEI-2018 251077, and the fund Impulso al desarrollo y conclusión de investigación con financiamiento externo (UAN-2017). Additionally, the authors thank Pas. Biol. Mar. Omar Cayetano-Monroy for his technical support in the development of the experiment.

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