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ZOOLOGY

Integrated multi-trophic culture of *Penaeus vannamei* with *Gracilaria domingensis* in biofloc system

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ABSTRACT. Co-cultures of the Pacific white shrimp *Penaeus vannamei* and seaweeds have been proposed as a promising and environmentally friendly way to reduce the levels of nitrogen and phosphorus in aquaculture wastewater. Therefore, the aim of this study was to evaluate the interaction between protein levels in feed as well as the stocking densities of the seaweed *Gracilaria domingensis* in an integrated culture with *P. vannamei* using biofloc system. To do so, two protein levels in feed (32% and 40% of crude protein) and four stocking densities (0, 2.5, 5.0, and 7.5 kg m^{-3}) of *G. domingensis* integrated with *P. vannamei* were evaluated at the secondary nursery phase, in factorial design (2-factor ANOVA). Shrimp (0.5 ± 0.05 g) were stocked at a density of 500 shrimp m^{-3} and reared for 42 days. During the experiment, water quality analysis was conducted, and at the end growth performance was evaluated. The water quality was not influenced by both factors, showing no significant differences. Regarding growth performance, the survival (86.2 ± 10.1%) and protein utilization efficiency rate (1.8 ± 0.3) did not differ among treatments. Feed conversion rate, weekly growth, and yield were influenced by protein percentage of the feed, presenting higher values when fed with 40% feed. However, the final mean weight of the shrimp integrated with the seaweed with 32% crude protein supply was similar to the monoculture treatment with 40% crude protein. In summary, the addition of *G. domingensis* in an integrated culture with *P. vannamei* did not affect shrimp performance.

Keywords: Protein; seaweed; growth performance; IMTA.

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Introduction

Biofloc systems are characterized by intensive oxygen supply with zero or minimal water exchange, reducing disposal of wastewater into adjacent ecosystems, and intensifying the animal stocking density (Samocha et al., 2017). Microbial flocs are composed by a heterogeneous group of microorganisms, including bacteria, algae, and zooplankton, as well as feces and other materials, which convert inorganic compounds into microbial biomass that can be consumed by target species in culture (Silva et al., 2013). The suitable relation between reducing inorganic compound levels and microbial biomass is based on the carbon-to-nitrogen (C:N) ratio (Samocha et al., 2017). However, parts of nitrogen (mainly in form of nitrate-N) and phosphorus-based compounds are accumulated in the culture water due to the minimal water exchanges, high densities, and the reuse of water between successive production cycles (Emerenciano et al., 2017).

The main route of entry of these inorganic compounds into rearing systems is through the continuous supply of feed (Samocha et al., 2017). Studies show that for every ton of shrimp produced, 29 to 73 kg of N and 12 kg of phosphorus (P) are generated in semi-intensive systems (Teichert-Coddington et al., 2000; Casillas-Hernández et al., 2006). In a biofloc-based system, it is estimated that 20 kg of N and 4.1 kg of P are produced, less than in other systems, as these nutrients are converted into bacterial flocs, thus enhancing usefulness (Silva et al., 2013). Nitrite-N and ammonia-N exhibit higher toxicity to shrimp compared to nitrate-N. In bioflocs and in clear water systems, prolonged exposure of *P. vannamei* to high concentrations of these compounds can reduce growth rates and impair gill and hepatopancreas histology, compromising overall performance (Melo et al., 2016; Furtado et al., 2015, 2016). Although the continuous accumulation of

P resulting from the supply of feed has been well studied, there are no reports of levels of P compounds considered toxic to shrimp (Samocha et al., 2017).

The crude protein content of the feed is the main source of residual N in shrimp farming, therefore, the N accumulation in the system will depend not only on the protein concentration of the feed, but also on its biological value, which confers different digestibility rates. Manipulation of C:N ratios in biofloc systems causes excretion rates of N compounds ranging from 50% (Avnimelech, 1999) to 80% (Emerenciano et al., 2017). Thus, applying 1 ton of feed with 35% crude protein can result in the release of 28 to 50.4 kg of nitrogen into the culture system.

In integrated multi-trophic systems, seaweeds can be used as a supplementary source of food for target species (Fleurence et al., 2012) and as a substrate for biofilm formation, as well as contribute to the control of N and P compounds (Tabarsa et al., 2012). *Gracilaria* spp. may contain from 6.4 to 37.6% protein and from 0.2 to 12.9% lipid (Øverland et al., 2019), acting not only as a source with high nutritional value, but also as a functional ingredient due to compounds with antioxidant and antimicrobial potential (Niu et al., 2019).

The main target of studying seaweeds in integrated systems is to improve water quality via phycoremediation. In addition, an increase in shrimp biomass of up to 15% is possible when using seaweeds in an integrated system with bioflocs (Attasat et al., 2013). Species of the genus *Gracilaria* stand out for their high efficiency in removing nitrogen and phosphorus compounds, with reported reductions of 35–100% for ammonia-N (Castelar et al., 2015), 26–84% for nitrite-N, 17–99% for nitrate-N, and 25–63.1% for phosphate compounds (Macchiavello and Bulboa, 2014; Brito et al., 2018a). In addition to the reduction of N and P contents, *Gracilaria* spp. may also influence the microbiological and phytoplanktonic community of cropping systems, due to allelopathic compounds that directly affect the growth of cyanobacteria (Elle et al., 2017).

Therefore, the present study aimed to analyze the effect of using different stocking biomass of the seaweed *Gracilaria domingensis* in integrated culture with Pacific white shrimp *P. vannamei* in a biofloc system when fed with different protein levels in the second nursery phase.

Materials and methods

Biological material

The experiment was conducted at the *Laboratório de Maricultura Sustentável* (LAMARSU), which is part of the *Departamento de Pesca e Aquicultura* of the *Universidade Federal Rural de Pernambuco* (UFRPE), using post-larvae of *P. vannamei* (1.00 ± 0.01 mg) purchased from Aquasul, Rio Grande do Norte, Brazil. The larvae were acclimated for 3 days until reaching post-larvae (pL) stage 10 (pL_{10} = ten days old) and cultured at a stocking density of 3,000 to 0.5 g of average weight (approximately 30 days).

Gracilaria domingensis was collected from a natural bank located at Pau Amarelo beach, Pernambuco, Brazil (07° 54’ 54.74” S, 034° 49’12.07” W). Sediments and fouling were carefully removed under running water, and then the specimens were acclimated in a 300 L tank for 5 days, through the daily inoculation of 15 L of water from a biofloc matrix tank. After acclimatization, the seaweeds were weighted and then stocked in the experimental units.

Preparation of biofloc

Biofloc was matured for 40 days, using commercial feed as nitrogen source and molasses as organic carbon source, maintaining a 4.8:1 C:N ratio (12:1 C:N ratio) (Samocha et al., 2007; Avnimelech, 2009). At the end of the maturation time, biofloc water had the following characteristics: 0.20 mg L^{-1} of total ammonia nitrogen (TAN), 0.40 mg L^{-1} of nitrite-N, 0.98 mg L^{-1} of nitrate-N, 182 mg L^{-1} of total suspended solids (TSS), 1.19 mg L^{-1} of orthophosphate, 30 g L^{-1} salinity, and 150 mg L^{-1} of $CaCO_3$.

Experimental conditions

The experimental design consisted of two factors: 1) *G. domingensis* biomass and 2) crude protein content in feed. Eight treatments corresponding to four levels of *G. domingensis* biomass, one of which stocked no seaweed at all (control), plus two crude protein levels were examined (Table 1). The experiment was performed in a completely randomized design with three replicates. Shrimp were stocked at a density of 500 shrimp m^{-3} (average weights of 0.5 ± 0.05 g) for 42 days. The seaweed (*G. domingensis*) was kept in meshes (0.5 cm) within the experimental units in the densities of each treatment (0 kg m^{-3}, 2.5 kg m^{-3}, 5.0 kg m^{-3}, and 7.5 kg m^{-3} wet weight). During culture, the wet biomass of seaweed of each experimental unit was measured.

Table 1. Experimental design of the Pacific white shrimp *Penaeus vannamei* integrated with *Gracilaria domingensis* fed with two protein levels.

	Gracilaria domingensis density (Kg m^{-3})			
Crude protein feed (%)	0	2.5	5.0	7.5
32[1]	0G32	2.5G32	5.0G32	7.5G32
40[2]	0G40	2.5G40	5.0G40	7.5G40

[1]32% crude protein, 8% lipids, 13% moisture, 4% crude fiber and 12% mineral material. [2]40% crude protein, 8% lipids, 13% moisture, 4% crude fiber and 12% mineral material.

The experimental units consisted of 60 L (50 L useful volume) polyethylene tanks for the rearing of shrimp. Each unit had a microperforated hose and a blower aeration system to keep the bioflocs in suspension and maintain oxygen above 5 mg L^{-1}, being kept under about 1,000 lux (natural irradiance and 12 hours photoperiod). No water exchange occurred during the experiment, and only freshwater was added to replace water lost by evaporation. Alkalinity control was performed by the addition of calcium hydroxide to maintain a level above 120 mg L^{-1}.

Shrimp were fed four times a day (8:00 am, 11:00 am, 02:00 pm, and 07:00 pm) with the commercial feed containing crude protein of the respective treatments, either 32% (0G32, 2.5G32, 5.0G32, and 7.5G32) or 40% (0G40, 2.5G40, 5.0G40, and 7.5G40). The amount of feed was adjusted according to the estimate of consumption and mortality, based on Van Wyk et al. (1999).

Water quality

The temperature and dissolved oxygen were measured twice a day (morning and afternoon) with an YSI 556 MPS meter in all experimental units. Settleable solids (SS, mL L^{-1}) were measured twice a week using Imhoff cone with 20 min sedimentation time (Avnimelech, 2009). Total suspended solids (TSS, mg L^{-1}) and nitrogen compounds (TAN, NO_2^--N, and NO_3^--N), alkalinity (mg L^{-1} $CaCO_3$), and phosphorus (PO_4^{3-}-P), were measured weekly, according to Grasshoff & Almgreen (1976), Golterman et al. (1978), MacKereth et al. (1978) and Felföldy (1987), respectively.

Shrimp performance

Shrimp weight was monitored weekly after 21 days of culture to determine growth and adjust the amount of feed offered. At the end of the experiment, mean final weight, biomass gain, weekly growth, specific growth rate (SGR), protein efficiency rate (PER), feed conversion ratio (FCR), survival, and yield were obtained.

Statistical analysis

The homogeneity distribution and normality of the data were assessed using Cochran and Lilliefors tests, respectively. Growth performance and water quality data were subjected to a two-factor analysis of variance (ANOVA), followed by Tukey's *post hoc* test for mean comparison ($p < 0.05$). All analyses were performed using Statistica 12 software.

Results and discussion

Mean temperature, dissolved oxygen, pH, and nitrogen and phosphorus compounds were maintained at optimal production levels for shrimp culture in all experimental conditions and in both two-nursery phases (Table 2). Throughout the cultures the levels of SS, TAN, NO_2^--N, NO_3^--N, PO_4^{3-}-P, and alkalinity did not exceed 22 mL L^{-1}, 0.8 mg L^{-1}, 2.37 mg L^{-1}, 5.1 mg L^{-1}, 2.74 mg L^{-1}, and 258 mg $CaCO_3$ L^{-1}, respectively, remaining among the suggested for shrimp nursery in biofloc technology (Samocha et al., 2017; Mendoza-López et al., 2017).

Some environmental parameters such as salinity, temperature, pH, and concentration of nitrogen and phosphorus compounds can affect the growth and survival of seaweeds (Susilowati et al., 2018). In addition, light availability can affect photosynthesis, limiting the growth of seaweed (Jiang et al., 2019). Therefore, an optimal seaweed stocking biomass may vary according to the shrimp stocking density and light availability. According to Susilowati et al. (2018), the optimal light intensity for a higher growth of *Gracilaria* spp. is around 3,500 lux. In this study, despite the light intensity, the high level of TSS may have reduced the light energy penetration into biofloc system, decreasing photosynthetic activity as well as nitrogen and phosphorus uptake. Moreover, during the experiment, small fragments of seaweed were observed in the water. The presence of these fragments may be related to the shearing of the algae caused by the high turbulence within the culture system. This turbulence is necessary to meet the high dissolved oxygen demands typical of intensive systems (Samocha, 2019).

Table 2. Water quality in the integrated culture of *Penaeus vannamei* and *Gracilaria domingensis* in nursery in biofloc system, over 42 days.

Variables	0G32	0G40	2.5G32	2.5G40	5.0G32	5.0G40	7.5G32	7.5G40	M	C	MxC
Temperature(°C)	30.5 ± 0.7	30.5 ± 0.7	30.6 ± 0.7	30.6 ± 0.9	30.7 ± 0.7	30.9 ± 0.6	30.6 ± 0.7	30.9 ± 0.7	ns	ns	ns
DO (mg L^{-1})	4.7 ± 0.6	4.6 ± 0.5	4.6 ± 0.1	4.8 ± 0.6	4.8 ± 0.7	4.6 ± 0.5	4.8 ± 0.7	4.7 ± 0.6	ns	ns	ns
pH	8.3 ± 0.1	8.2 ± 0.1	8.2 ± 0.1	8.2 ± 0.1	8.2 ± 0.2	8.2 ± 0.1	8.2 ± 0.1	8.2 ± 0.1	ns	ns	ns
Salinity	35.1 ± 1.0	35.6 ± 1.0	35.2 ± 1.0	35.2 ± 1.0	34.4 ± 1.5	35.5 ± 1.0	35.2 ± 1.0	35.3 ± 0.9	ns	ns	ns
SS (mL L^{-1})	14.0 ± 5.5	15.2 ± 3.4	14.8 ± 3.0	13.5 ± 4.1	16.2 ± 3.7	15.8 ± 3.8	17.7 ± 4.3	11.9 ± 4.5	ns	ns	ns
TAN (mg L^{-1})	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.3	0.25 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.3	ns	ns	ns
NO_2-N (mg L^{-1})	1.16 ± 1.2	1.0 ± 0.9	1.0 ± 0.9	1.1 ± 1.1	1.08 ± 0.9	0.8 ± 0.27	0.9 ± 0.7	1.1 ± 1.0	ns	ns	ns
NO_3-N (mg L^{-1})	3.4 ± 1.5	3.1 ± 2.0	3.4 ± 1.5	3.4 ± 1.3	3.78 ± 1.2	3.4 ± 01.4	2.8 ± 1.3	2.8 ± 1.5	ns	ns	ns
$CaCO_3$ (mg L^{-1})	113.3 ± 30.9	113.4 ± 27.3	119.2 ± 32.8	115.9 ± 31.7	111.7 ± 34.2	116.7 ± 32.6	108.2 ± 29.9	115.1 ± 33.2	ns	ns	ns
PO^{-3}_4-P	1.8 ± 0.2	2.1 ± 0.3	2.2 ± 0.6	2.2 ± 0.5	2.0 ± 0.2	2.0 ± 0.3	2.1 ± 0.4	2.1 ± 0.3	ns	ns	ns

The data correspond to the mean of values sampled over the entire experimental period ± standard deviation by treatments. Result of the factorial analysis of variance (ANOVA- Factorial) representing factor 1 is density of seaweed *G. domingensis* in Kg m^{-3} by M, factor 2 is percentage of crude protein in the ration by CP and the interaction between factors by M x CP. ns - not significant. G32-0: Without seaweed and feed with 32% CP; G40-0: Without seaweed and feed with 40% CP; G32-2.5: With *G. domingensis* (2.5 kg m^{-3}) and feed with 32% CP; G40-2.5: With *G. domingensis* (2.5 kg m^{-3}) and feed with 40% CP; G32-5.0: With *G. domingensis* (5.0 kg m^{-3}) and feed with 32% CP; G40-5.0: With *G. domingensis* (5.0 kg m^{-3}) and feed with 40% CP; G32-7.5: With *G. domingensis* (7.5 kg m^{-3}) and feed with 32%. CP; G40-7.5: With *G. domingensis* (7.5 kg m^{-3}) and feed with 40% CP. Abbreviations: DO - Dissolved oxygen; TAN - Total ammonia nitrogen; SS - Settleable solids; $CaCO_3$ - Alkalinity.

Brito et al. (2018a) evaluated the bioremediation potential of *G. birdiae* (at 2.5 kg m^{-3} in wet weight) in effluents from juvenile *P. vannamei* culture in a biofloc system and found no significant changes in total ammonia nitrogen, nitrate, or orthophosphate concentrations after 96 hours. Furthermore, Zhou et al. (2021), when evaluating the bioremediation of *P. vannamei* culture effluent with seaweed (*Gracilaria lichenoides*) at distinct densities (0.5, 2, and 4 kg m^{-3}), observed no differences in nutrient removal rate among the tested densities other than from the control. Conversely, Samocha, Fricker, Ali, Shpigel, and Neori (2015), using a recirculating aquaculture system based on *Gracilaria* sp. (at 2.96 kg m^{-3}), reported a reduction in the concentrations of inorganic dissolved compounds.

The growth performance of the shrimp in the secondary nursery phase and the effect of the analyzed factors are summarized in Table 3. Our study found that varying the percentage of crude protein in the shrimp's feed influenced their mean final weight. When we analyzed the group averages at 32% and 40%, they were significantly different, reaching 2.94 g and 3.71 g, respectively. Regarding the influence of *G. domingensis* biomass on the distinct groups, the shrimp from the treatments with 5.0 kg m^{-3} density (3.51 g) had a higher average weight than the treatments without the presence of seaweed. However, when analyzing the effect of the combination of factors, it was observed that all treatments were similar to each other.

Table 3. Zootechnical development indices of *Penaeus vannamei* cultured with seaweed *Gracilaria domingensis* in a secondary nursery using a biofloc system.

Treatments	Survival (%)	Final weight (g)	SGR (% day^{-1})	EPR	FCR	Yield (kg m^{-3})
0G32	88.0 ± 10.6	2.7 ± 0.2	4.3 ± 0.2	1.6 ± 0.3	1.9 ± 0.3	1.2 ± 0.1
0G40	86.7 ± 10.1	3.4 ± 0.0	4.6 ± 0.1	1.7 ± 0.2	1.5 ± 0.2	1.5 ± 0.2
2.5G32	94.7 ± 2.3	2.9 ± 0.2	4.1 ± 0.1	1.9 ± 0.2	1.7 ± 0.2	1.4 ± 0.1
2.5G40	92.0 ± 6.9	3.8 ± 0.2	5.0 ± 0.2	2.1 ± 0.1	1.2 ± 0.0	1.7 ± 0.1
5.0G32	88.0 ± 10.6	3.2 ± 0.3	4.3 ± 0.3	1.9 ± 0.2	1.6 ± 0.2	1.4 ± 0.1
5.0G40	81.3 ± 8.3	3.8 ± 0.4	4.9 ± 0.2	1.8 ± 0.3	1.4 ± 0.2	1.6 ± 0.2
7.5G32	90.7 ± 10.1	2.9 ± 0.1	4.1 ± 0.0	1.8 ± 0.2	1.8 ± 0.2	1.3 ± 0.1
7.5G40	78.7 ± 12.9	3.8 ± 0.2	4.9 ± 0.2	1.7 ± 0.4	1.5 ± 0.4	1.5 ± 0.2
S	ns	ns	ns	ns	ns	ns
CP	ns	*	*	ns	*	*
MxCP	ns	ns	ns	ns	ns	ns

Means and standard deviations of values distributed by treatment. Result of the factorial analysis of variance (ANOVA- Factorial) representing factor 1 is density of seaweed *G. domingensis* in Kg m^{-3} by M, factor 2 is percentage of crude protein in the ration by CP and the interaction between factors by M x CP. * - $p < 0.05$; ns - not significant. G32-0: Without seaweed and feed with 32% CP; G40-0: Without seaweed and feed with 40% CP; G32-2.5: With *G. domingensis* (2.5 kg m^{-3}) and feed with 32% CP; G40-2.5: With *G. domingensis* (2.5 kg m^{-3}) and feed with 40% CP; G32-5.0: With *G. domingensis* (5.0 kg m^{-3}) and feed with 32% CP; G40-5.0: With *G. domingensis* (5.0 kg m^{-3}) and feed with 40% CP; G32-7.5: With *G. domingensis* (7.5 kg m^{-3}) and feed with 32%. CP; G40-7.5: With *G. domingensis* (7.5 kg m^{-3}) and feed with 40% CP. Abbreviations: SGR – Specific growth rate; EPR – Efficiency protein rate; FCR – Feed conversion ratio.

Correia et al. (2014) reported similar findings regarding the effect of protein concentration in a primary nursery using a biofloc system. They observed that shrimp fed with a 40% crude protein diet achieved higher final weight compared to those fed a 30% crude protein diet, demonstrating that despite biofloc being a supplemental feed source, shrimp fed a diet with a protein content near 40% exhibited better growth performance. Brito et al. (2018b), when analyzing the zootechnical development of *P. vannamei* juveniles integrated with the seaweed *G. birdiae* fed with 40% and 32% protein, also observed significant differences between the average final weight of the shrimp when analyzing the factors (integrated or monoculture system with *G. birdiae* and crude protein content) separately, as well as the findings of the present study in the secondary nursery stage. According to reports by Fourooghifard et al. (2018) and Gao et al. (2022), evaluating the integration of *P. vannamei* with *Gracilaria* sp. and *Ulva linza*, respectively, the presence of seaweed resulted in a higher average final weight of the shrimp.

The difference between monoculture and integrated culture with *Gracilaria* sp. could be attributed to the utilization of seaweed as a supplementary food source and substrate for colonization by microorganisms (biofilm), which may also have contributed to the shrimp's diet. A biofilm is an organic matrix that adheres to a submerged natural or artificial substrate and is colonized by a microbial community (Hall-Stoodley et al., 2004). This can be an efficient option for supplementing the feed supply of *P. vannamei* (Santos et al., 2020).

At the end of the trial, seaweed density decreased in all treatments (Figure 1), between 19.2% (5.0G40) and 38.2% (7.5G32), suggesting its consumption by shrimp. Small seaweed fragments were observed in the water column, allowing their aggregation to bioflocs and increasing consumption, thereby reducing FCR and enhancing animal growth, as reported by Brito et al. (2014) and Mangott et al. (2020). Biomass of *Gracilaria* was reduced, indicating culture tanks did not present ideal conditions for its growth, therefore, its biofilter function may have been limited, causing a reduction in biomass and the release of more nutrients into the medium. The fragmentation of seaweed can be linked to the high turbulence in the cultivation system, which is due to the continuous supply of dissolved oxygen when using biofloc technology.

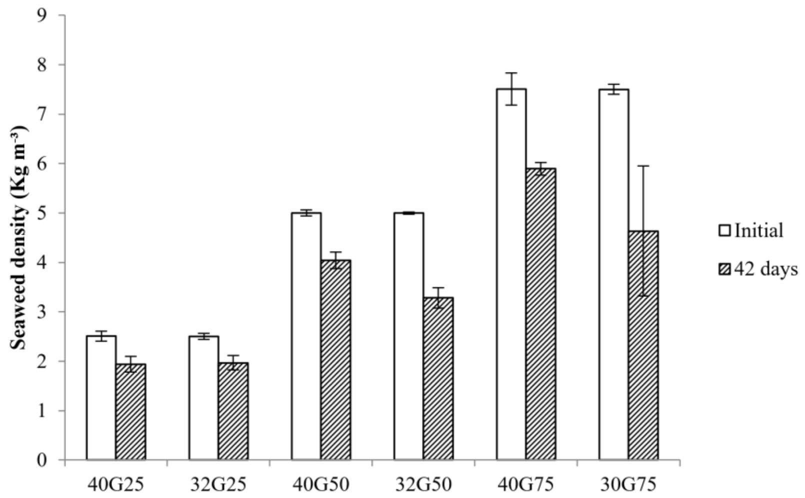

Figure 1. Wet weight and density of seaweed *Gracilaria domingensis* in integrated culture with *Penaeus vannamei* in biofloc system.

As a result, it is expected that *Gracilaria* species will serve as a similar supplementary food source for shrimp, enabling a reduction in the protein percentage of the feed without compromising the animal's biomass gain. Additionally, it was observed that the protein percentage had an influence on the weekly growth results, with higher values associated with the feed containing a higher protein concentration. As a result, there were significant differences in weekly growth averages, with a mean growth of 0.89 g week^{-1} for treatments with 32% crude protein and 1.10 g week^{-1} for treatments with 40% crude protein. However, there was no observed interaction between the two factors analyzed. The crude protein percentage in the feed also had an impact on the specific growth rate of the shrimp, as with the weekly growth. In the distinct groups of 32 and 40%, respective means of 4.2 and 4.8% were significantly different. Final average weights of 2.94 g and 3.71 g were obtained for animals fed with 32 and 40% protein feed, respectively, in relation to the influence of *Gracilaria domingensis* density on the distinct groups without seaweed. No isolated influence of seaweed density was observed. When analyzing the interaction effect between factors, a significant influence was observed on specific growth rates in the secondary nursery, with the highest values being associated with treatments using 40% crude protein feed in the presence of seaweed.

When analyzing the effect of different nutrient inputs for feeding *P. vannamei* post-larvae in a primary biofloc nursery system, Correia et al. (2014) achieved maximum specific growth rates of 11.24% per day, with the highest values being attributed to the use of food with high protein content. The same was observed in the present study, which resulted in higher specific growth rates when using a diet with 40% protein. Additionally, the combined effect of the factors was observed, with the highest specific growth rate values being linked to the use of a high protein content diet. However, the other treatments using a 32% protein diet did not differ from the specific growth rate of monocultures using a 40% protein diet. This difference may be related to the total trial time and final weight, compared to the previously cited study.

In a study on the use of artificial substrates for juvenile *P. vannamei* culture, Ferreira et al. (2016) found that increasing substrate area led to higher average final weights and specific growth rates compared to the control group without substrates. When submerged in seawater, any surface can act as a substrate for biofilm formation (Callow and Callow, 2002), with bacteria being its initial colonizers (Busetti et al., 2017). Therefore, the presence of seaweed enables the formation of a bacterial biofilm that can be used as a supplementary food source for *P. vannamei*.

No isolated effect or interaction of the analyzed factors was observed on the protein efficiency rate. The feed conversion rate was influenced by the percentage of feed protein, showing a significant difference, and the highest FCR values were attributed to treatments using 32% crude protein. According to Tantikitti et al. (2016), the quality and quantity of protein used in the feeding of *P. vannamei* juveniles can affect their growth performance. In a comparison of the effects of using feeds with different protein levels in clear water and biofloc systems, Brito et al. (2018b) obtained similar protein efficiency ratios to those of the present study. In addition, Correia et al. (2014) did not observe a significant difference in protein efficiency ratios when analyzing the effect of using feeds with different protein levels for *P. vannamei* post-larvae reared in a biofloc system.

Van Wyk et al. (1999) recommended the use of feeds with protein levels ranging from 50% to 45% for *P. vannamei* weighing between 2 mg and 1 g. According to Jackson et al., (2003), protein is the most expensive nutrient in shrimp feeds, and reducing its concentration in the feed can lead to negative effects on the animal's growth performance. However, when analyzing the zootechnical indices of *P. vannamei* juveniles cultured in an integrated system with a *Gracilaria* seaweed in a no-water exchange system, Fourooghifard et al. (2018) attributed the best results in feed conversion ratio, specific growth rate, weekly growth, and survival rate to the treatment with lower densities of 25 shrimp m^{-2} and 400 g of *G. corticata* m^{-2}. Similarly, Brito et al. (2018b) stated that the presence of *G. birdiae* in the culture of *P. vannamei* juveniles in a biofloc system may be a strategy that enables the use of feeds with lower protein content, favoring the animal's growth parameters.

According to Fourooghifard et al. (2018), it is possible to minimize damage to the growth of *P. vannamei* resulting from high stocking rates by using seaweed of the genus *Gracilaria*, which can serve as a natural substrate for the colonization of microorganisms as well as a potential source of supplementary food or shelter for the shrimp.

The yield data were influenced by the percentage of crude protein, presenting respective averages of 1.3 and 1.5 kg m^{-3} for the use of 32% and 40% of crude protein. When analyzing the effect of combining different densities of *P. vannamei* and the seaweed *G. corticata* on the final biomass of shrimp fed with 35% of crude protein feed, Fourooghifard et al. (2018) observed that increasing the stocking density of seaweed led to an increase in the final biomass of *P. vannamei*, which resulted from the use of seaweed as a food supplement. Similarly, Brito et al. (2018b) and Campos et al. (2019) also reported a positive effect of adding seaweed in intensive systems compared to shrimp monoculture. The disparity between the previously mentioned findings and the current study could be attributed to the seaweed's growth in the system over the culture period, as indicated by Fourooghifard et al. (2018). Even though the impact of seaweed was not observed in isolation, the treatments did not differ from one another when analyzing the interaction between the factors. Hence, the utilization of feed with 32% protein, combined with the presence of seaweed, makes it feasible to obtain productivity levels comparable to those obtained with 40% crude protein feed.

Conclusion

The interaction between *G. domingensis* biomass and feed protein levels did not significantly affect growth performance indicators such as final weight, protein efficiency ratio, feed conversion ratio, or productivity. Therefore, integrating *G. domingensis* into a biofloc system allows the use of feed with 32% crude protein without compromising the growth performance of *Penaeus vannamei*.

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