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Use of Lithothamnium sp. (Algen® Oceana) in Penaeus vannamei culture

Uso do *Lithothamnium* sp. (Algen® Oceana) no cultivo de *Penaeus* vannamei

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ABSTRACT

The process of preparing the soil for ponds is a very important phase of production. The selection of the type of limestone used in the pH correction process will directly influence the quality of the soil, water, natural food production and the development of *Penaeus vannamei*. The objective of this study was to evaluate a substitute for one of the types of limestone used in pond liming, originated from the limestone algae *Lithothamnium* sp., commercially named Algen® (Oceana, Tutóia, Maranhão, Brazil), verifying its efficiency in relation to dolomitic limestone. The study was conducted at the shrimp farm Monólitos Aquacultura Ltda (Banabuiú, Ceará, Brazil). The monitoring and evaluation of the used products, soil physical-chemical properties, physical-chemical and biological analyses of the water, and the zootechnical performance of the shrimp were performed during two production cycles between November 2017 and July 2018 in three ponds with an area of 1.0 ha. The results showed that the use of Algen® showed similar action to the parameters obtained with the use of dolomitic limestone, both in terms of soil and water quality aspects and for the zootechnical performance of *P. vannamei*. However, a better zootechnical performance of *P. vannamei* was observed when Algen® was used in the 500 kg/ha concentration.

Keywords: Shrimp farming, Liming, Dolomitic limestone, Lithothamnium sp.

RESUMO

O processo de preparação do solo de viveiros é uma etapa da produção muito importante. A escolha do tipo de calcário utilizado no processo de correção do pH irá influenciar diretamente

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na qualidade do solo, da água, na produção de alimento natural e no desenvolvimento de *Penaeus vannamei*. O objetivo do presente trabalho foi avaliar um substituto para um dos tipos de calcário utilizado na calagem de viveiros, originário da alga calcária *Lithothamnium sp.*, denominado comercialmente de Algen® (Oceana, Tutóia, Maranhão, Brasil), verificando sua eficiência em relação ao calcário dolomítico. O estudo foi realizado em uma fazenda de camarão Monólitos Aquacultura Ltda (Banabuiú, Ceará, Brasil). Para o monitoramento e avaliação dos produtos utilizados, análises físico-química de solo, físico-química e biológica da água e do desempenho zootécnico do camarão foram realizados durante dois ciclos de produção, entre novembro de 2017 e julho de 2018, em três viveiros com área de 1,0 ha. Os resultados evidenciaram que o uso de Algen® apresentou ação similar aos parâmetros obtidos com o uso do calcário dolomítico, tanto em relação aos aspectos de qualidade de solo e água como para o desempenho zootécnico de *P. vannamei*. Contudo, verificou-se um melhor desempenho zootécnico de *P. vannamei* quando Algen® foi utilizado na concentração de 500kg/ha.

Palavras-chave: Carcinicultura, Calagem, Calcário dolomítico, Lithothamnium sp.

1 INTRODUCTION

Liming, a conventional practice used in agriculture, is also widely used in shrimp farming with the same intention of reclaiming the soil. This practice consists of applying calcium and magnesium to the soil in order to correct its acidity (QUEIROZ; BOEIRA, 2006). The neutralization of acids and bases provides macro and micronutrients that were stabilized in the environment, besides accelerating the mineralization of organic matter. The caustic reaction eliminates most microorganisms, especially pathogens. However, excessive liming can cause a decrease in the availability of nutrients essential to phytoplankton development, such as the precipitation of phosphorus as calcium phosphate (BOYD, 1995).

Soil liming can be done with virgin lime, limestone, and dolomitic limestone and the amounts applied depend on the result of soil pH mapping (VINATEA; MALPARTIDA; ANDREATTA, 2004). This process directly affects the total alkalinity of the water when the pond is filled, being defined as the sum of the titratable bases, especially carbonates and bicarbonates, increasing the water's buffering power, resulting in the water's capacity to resist pH changes (BARBIERI-JUNIOR; OSTRENSKY-NETO, 2002). Usually, the most common substances found in alkaline water are carbonates (CO_3^{-2}), bicarbonates (HCO_3^{-}) and hydroxides (OH^{-}). The total alkalinity of water is mainly derived from the dissolution of soil limestone so that the concentration of total alkalinity is determined mainly by soil characteristics (QUEIROZ; BOEIRA, 2006).

According to Queiroz and Boeira (2006), the climate is also a factor that can influence alkalinity. For instance, ponds located in arid regions have soils with higher total alkalinity

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than ponds located in humid regions. In aquaculture ponds, liming aims to maximize productivity and improve environmental sustainability, as well as neutralize the surface layer of sediment from the bottom of the ponds and increase the total alkalinity and total hardness of the water. The soil acidity of the ponds should be corrected until the pH reaches values between 7.0 and 8.0 while the concentrations of total alkalinity and total water hardness should be raised above 20 mg/L (BOYD; TUCKER, 1998).

In aquatic ecosystems, primary productivity is strongly influenced by alkalinity due to its performance in chemical and physiological processes. Liming procedures improve the quality of ponds water leading to an increase in alkalinity and calcium, promoting changes in the biotic community (ROJAS *et al.*, 2004). When in the early stages of their life cycle, crustaceans and fish feed mainly on natural food that consists of plankton, basically represented by phytoplankton (microalgae) and zooplankton (rotifers, copepods, cladocerans) (FERREIRA, 2009). The availability of these organisms in ponds can lead to a higher performance of the cultivated animals, maximizing the production in the culture. Microalgae, especially diatoms, are essential for proper animal nutrition in extensive and semi-intensive shrimp farming.

Lithothamnium sp. is an alga that belongs to the Coralineacea family, of the Rhodophyta or red group, presenting a calcareous aspect, absorbing from the environment mainly calcium and magnesium carbonate (MELO *et al.*, 2006). They are obtained from the marine environment through several manual or mechanical methods. After extraction, the process of washing, drying, and grinding occurs to obtain the powder, not going through any chemical process.

According to Dias (2000), *Lithothamnium* sp. is composed of calcium carbonate in addition to more than 20 micronutrients such as magnesium, nickel, boron, zinc, copper, iron, molybdenum, strontium and selenium. This compound has been used for more than 200 years in agriculture as a fertilizer and as a supplement to animal nutrition, having a great potential for expansion in several areas, such as aquaculture (MELO *et al.*, 2006). The limestone produced by the extraction of *Lithothamnium* sp. can be called biogenic or marine biodentritic limestone due to its high concentration of calcium carbonate. It is used for soil reclaiming and fertilization in agriculture and may be an alternative in pH control in aquaculture ponds (GOETZ, 2008; COSTA-NETO *et al.*, 2010).

On the coast of Maranhão, the calcified algae deposits are located in Tutóia (Maranhão, Brazil) in an area of 11,000 hectares with a depth of between 18 and 30 meters. Its extraction

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is done by dredging, and no chemical additives are used during its processing, preserving the physical-chemical, biological and nutritional parameters. Being it considered an organic product (LÓPEZ-BENITO, 1963).

Researches regarding the use of *Lithothamnium* sp. in aquaculture are scarce. Thus, there is a need to provide scientific information on the subject. Due to this scarcity, the present research aimed to study and evaluate the use of *Lithothamnium* sp. (Algen®, Oceana) as a replacement of dolomitic limestone in a *P. vannamei* culture.

2 MATERIALS AND METHODS

The research was conducted at Monólitos AquaculturaLtda (Banabuiú, Ceará, Brazil). The property has 15.15 ha of water mirror distributed in 17 ponds. A water recirculation system is used, with a 1.83 ha sedimentation lagoon where the harvest effluent is released and then, after treatment, reused.

In the present study, 3 ponds with an area of approximately 1.0 hectare were used during 2 production cycles. After drying the ponds by exposure to the sun for 7 days, 1,000 kg of dolomitic limestone (Control Group) was applied on the first pond, 250 kg of Algen® (Treatment 1, T1) on the second and 500 kg of Algen® on the third (Treatment 2, T2). The products were incorporated into the soil by plowing the ponds using a motorized plow. For a greater efficiency in the disinfection process, active chlorine (20 ppt, based on 2 kg/hectare) was applied in the humid areas and feeding structures.

The water supply (salinity of 0.5 ppt) of the ponds happened 2 days after the incorporation of the products and collection of soil for analysis. It was done through the supply gate with water from artesian wells or the recirculation system. After this procedure, a process to promote a natural food bloom (plankton) was performed using a fertilization based on 50 kg of rice bran, 2.5 kg of sodium bicarbonate and 0.75 kg of BM-PRO probiotic (Biotrends Ltda, Fortaleza, Ceará, Brazil), diluted in 875 L of water and after activation for 48 hours. After 10 days of preparation, the ponds received the post-larvae of *Penaeus vannamei* (700,000 PLs per pond) from CELM Aquicultura S.A. (Aracati, Ceará, Brazil), with an initial average weight of 0.02 g.

For soil pH analysis, N-subsamples of substrate were collected in several points of each pond. The samples were homogenized in plastic bags, which resulted in 3 samples for each pond, divided in: the region near the supply gate, the region near the feeding trays and the region near the discharge channel. With the use of a soil pH meter, the determination was

made before the application of the products, 48h after application and at the end of the cultivation.

During the study period, daily monitoring of the dissolved oxygen level and temperature was performed with a multiparameter probe AK87. For the other water quality parameters (pH, salinity, total alkalinity, total hardness, calcium, magnesium, nitrate, nitrite, total ammoniacal nitrogen, phosphate, silica, apparent color, and chlorophyll), three collections were performed during the experiment. Water samples were collected and analyzed at the Laboratory of Marine and Applied Geology, belonging to the Department of Geology, Science Center, Federal University of Ceará, (Fortaleza, Ceará), following the methodologies described by Aminot and Kérouel (2004) and APHA (2012).

The plankton collection was conducted at the beginning and end of the cultivation, around 10:00 h, at eight different points of the farm, one collection point in each of the six ponds used in the experiment, with the sampling performed near the drainage systems and at two more points in the supply gate.

For qualitative analysis, 200 mL were collected from each sample and concentrated with the aid of a plankton net of 20 μ m. Then they were bottled and fixed with formalin buffered with sodium tetraborate. Identification was performed through light-field optical microscopy with a binocular microscope, using keys with the following classification keys: Anagnostidis; Komárek (1986, 1988, 1998), Bourrelly (1985), Bicudo; Menezes (2006).

For quantitative analysis, 1,000 mL were collected from each sample in an amber glass bottle, fixed with acetic Lugol, in the proportion of 5 mL/L for phytoplankton samples. For zooplankton sampling, a volume of 60 L of the sample was filtered in a plankton net with 60 μ m, collectingthe material retained in the collecting cup at the end of the filtration process and later recording the final volume. The phytoplankton count was performed by inverted microscopy, from the sediment, with a Sedgewick-Rafter chamber, using field subdivisions according to the Poisson distribution, with a 95 ± 20% confidence interval, after concentration of the samples by sedimentation in a 1,000 mL specimen for 24 hours. The zooplankton was quantified by the scanning methodology using the Sedgewick-Rafter chamber with an inverted microscope.

The quantity and quality of the planktonic community during the cultivation days were compared among the different collection points to observe possible changes among the analyzed groups. All the analyses were conducted at the Ambiental Análises e Consultoria LTDA laboratory (Fortaleza, Ceará).

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The shrimps were fed 4 times a day with extruded commercial feed, containing 40% (35 first days of cultivation, GuabiTech Inicial, Guabi®, São Paulo, Brazil) and 35% crude protein (from the 36th day until the end of cultivation) (PotiGuaçu 35, Guabi®, São Paulo, Brazil). Feed rates decreased ranging from 5.0 to 3% body weight/day.

Weekly, shrimps were sampled to assess growth in weight. To this end, 200 shrimps in each pond were caught with a cast net at different points of the pond and weighed. After each sampling, the amount of fermented soybean supplied was adjusted to the average weight and biomass of each tank. At the end of the 99 days of culture, the shrimps were captured and the final survival (%), the final weight (g), daily weight gain (average final weight - initial average weight/culture days, g/day), absolute growth rate (AGR, g/shrimp/week), specific growth rate (SGR = (ln final weight - ln initial weight)/days x 100, %/day), productivity (net biomass/pond area, kg/ha), and feed conversion ratio (feed consumption/net biomass) for each pond and treatment were calculated.

All the data are presented in mean \pm standard deviation, to verify significant differences between the means of the two groups analyzed, the data were submitted to Student's t-test, with the level of significance *p*<0.05. PAST3.20 and Excel 2010 software were used to perform the tests.

3 RESULTS

Table 1 shows the pH values of soil samples obtained from the ponds during the experimental procedure. The monitoring of water quality during the two production cycles for the control group and the two treatments are expressed in Table 2. The qualitative analysis of phytoplankton and its representation by taxons are described in Table 3. The zooplankton density in experimental ponds and its respective taxons are represented in Tables 4 and 5, respectively. Information on the zootechnical performance of *Penaeus vannamei* during the experimental period is presented in Table 6.

4 DISCUSSION

At the end of the culture, it was verified that the pH values of the soil both for the control group and for both treatments were close to neutrality, with no statistical difference (p>0.5), the values being within the range suggested by Body and Tucker (1998).

The monitoring of water quality during the experiment showed that there was no statistical difference (p>0.05) between the treatments, regardless of the production cycle.

Dissolved oxygen (DO) values remained within the optimal range for this parameter, ranging from 5 to 10 mg/L O₂ (BOYD; TUCKER, 1998; BARBIERI-JUNIOR; OSTRENSKY-NETO, 2002; SLA, 2009), and the pond aerators were activated during the night period, running until 07:00 h of the following morning. Similarly, pH values varied between 7.55 and 8.16, while salinity between 1.33 and 2.01 ppt, which is a characteristic of oligohaline waters, and for both cases, there were no statistical differences (p>0.05) (BOYD; TUCKER, 1998; WYK *et al.*, 1999; SLA, 2009).

In the first production cycle, the alkalinity ranged from 129.3 to 117.2 mg/L CaCO₃ with T1 presenting an alkalinity of 224.2 mg/L CaCO₃ after 45 days of cultivation. In the second production cycle, the values varied between 50.5 and 111.1 mg/L CaCO₃ during most of the culture, being above the minimum dosage suggested for shrimp culture which is 75 mg/L CaCO₃ (BOYD, 2002). Water hardness is related to the amount of calcium and magnesium available (SIPAÚBA-TAVARES, 1995), and during the experiment, the hardness values remained above the minimum recommended levels (> 150 mg/L CaCO₃). The calcium values varied between 54.4 and 90.9 mg/L Ca⁺² staying below the desirable concentration which is 100 mg/L Ca⁺² (WYK *et al.*, 1999; NUNES, 2002). However, the magnesium concentrations, varying between 53.5 and 79.7 mg/L Mg⁺², remained above the minimum recommended levels which is 50 mg/L Mg⁺² (NUNES, 2002).

The concentrations of ammonia, nitrite, and nitrate remained always below 0.001 mg/L, and it was not possible to quantify them, as they were below the detection level of laboratory analyses. For the culture of *Penaeus vannamei*, the toxic concentration of these compounds is normally above 2.0-10 mg/L (BARBIERI-JUNIOR; OSTRENSKY-NETO, 2002). For the other studied parameters (silica, turbidity, iron, phosphorus, phosphate, sulfate, and sulfide), no toxic levels were found or values were in the normal recommended range (BOYD, 2000; SÁ, 2012).

The quali-quantitative analysis of phytoplankton showed that Cyanophytes represent a total of 97.2% of the microalgae found, followed by Bacillariophytes with 1.4%, Chlorophytes with 1.3%, while Euglenophytes, Cryptophytes, and Dinophytes represent around 0.1%, corroborating with the study of Alonso-Rodriguez and Paez-Osuna (2003) and Fonseca (2006). Regarding the diversity of organisms, the greatest contribution of species was for the Chlorophyta group with 40,5%, followed by Cyanophyta with 38,1%, Bacillariopyta with 9,5% and the Euglenophyta, Cryptophyta, and Dinophyta groups with 11,9%.

According to the quali-quantitative analysis of the zooplankton, the Rotifer group has the highest representation of the samples with 78.4%, followed by Crustacea 18.8% and Protozoa 2.8%. Of the 14 zooplankton identified at the genus or species level, 6 belonged to the rotifer group, which is equivalent to 42.9%, 5 to Protozoa 35.7%, and 3 to Crustacea 21.4%. A statistical difference (p<0.05) was observed for the zooplankton density for the Rotiferous and Crustacean groups when T1 and T2 treatments were compared with the control group, but not for the Protozoa group.

Regarding zootechnic performance, there was no significant difference (p>0.05) between the control group and Algen® treatments, with values similar to those observed by Nunes (2002), Spanghero *et al.* (2008), and Nunes, Madrid and Andrade (2011).

5 CONCLUSIONS

The results found in this study recommend the use of Algen® as a dolomitic limestone substitute in liming procedures on *Penaeus vannamei* cultures since the analyzed data were similar both for the control groups and for Algen® treatments at 250 and 500 kg/ha doses. Despite the statistical similarity, in the Algen® treatment at 500 kg/ha dose, the highest average weight (11.7 g), the highest productivity (5,176 kg/ha), indicating a higher revenue, and the lowest FCR (1.70:1) were found, resulting in a lower feed cost.

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Table1 Soil pH values during Penaeus vannamei farming.				
	Т	Teatt		
Parameter	Dolomitic limestone	Algen®	Algen®	Test t
	(1,000 kg/ha)	(250 kg/ha)	(500 kg/ha)	р
pH before product application	7.3 ± 0.07	6.8 ± 0.42	6.8 ± 0.28	1
pH 48h after product application	7.6 ± 0.07	6.4 ± 1.84	6.9 ± 0.28	0.37
pH after the end of the culture	7.4 ± 0.14	7.3 ± 0.57	7.5 ± 0.49	0.93

		Treatment		
Parameter	Dolomitic limestone	Algen®	Algen®	Test t
	(1,000 kg/ha)	(250 kg/ha)	(500 kg/ha)	р
Dissolved oxygen (mg/L)	8.1 ± 0.8	9.4 ± 2.1	8.3 ± 1.1	0.690
pH	7.5 ± 0.3	8.2 ± 0.1	7.8 ± 0.4	0.275
Salinity	1.33 ± 0.02	2.01 ± 0.47	1.96 ± 0.55	0.338
Alkalinity (mg/L CaCO ₃)	120.2 ± 27.2	117.6 ± 34.9	104.6 ± 49.3	0.911
Hardness (mg/L CaCO ₃)	370.5 ± 36.1	492.0 ± 131.5	464.0 ± 135.8	0.580
Calcium (mg/L Ca ⁺²)	59.0 ± 11.6	64.0 ± 22.6	60.6 ± 21.8	0.966
Magnesium (mg/L Mg ⁺²)	53.5 ± 10.7	79.7 ± 18.0	75.0 ± 19.5	0.329
TAN (mg/L N-NH _{3,4})				
Ammonia (mg/L NH ₃)				
Nitrate (mg/L N-NO ₃ ⁻)				
Nitrite (mg/L N-NO ₂ ⁻)				
Silica (mg/L SiO ₂)	13.7 ± 1.6	2.2 ± 0.2	6.6 ± 6.5	0.124
Turbidity (UNT)	89.5 ± 12.0	120.2 ± 8.8	122.5 ± 14.1	0.115
Iron (mg/L Fe)	1.35 ± 0.14	1.25 ± 0.14	1.20 ± 0.21	0.695
Phosphorus (mg/L)	0.16 ± 0.02	0.17 ± 0.01	0.16 ± 0.01	0.809
Phosphate (mg/L P-PO ₄ - ³)				
Sulfate (mg/L SO4 ⁻²)	68.9 ± 22.8	37.0 ± 1.7	26.0 ± 3.2	0.097
Sulfide (mg/L S ⁻²)	0.025 ± 0.040	0.047 ± 0.025	0.150 ± 0.021	0.192

Table 2 Water quality parameters in *Penaeus vannamei* culture.

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Treatment				
	Dolomitic	Algen®	Algen®	
Taxon	limestone (1,000 kg/ha)	(250 kg/ha)	(500 kg/ha)	
Cyanophyta				
Anabaenopsis sp.	+++	++++	++++	
Aphanizomenon sp.		+	+	
Aphanocapsa sp.	++	+++	++	
Chroococcus sp.	+++			
Coelomoron sp.	+++	++++	+++	
Cylindrospermopsis sp.	+++	+++	+++	
Geitlerinema sp.	++++	++++	++++	
Merismopedia sp.	+++	+++	++	
Microcystis sp.	+	++	+++	
Synechocystis sp.	++++	+++	+++	
Oscillatoria sp.	+++	+++	++++	
Pseudanabaena sp.	++	++++	++++	
Spirulina sp.	+++			
Chroococcales	++++	++++	++++	
Nostocales		++++		
Pseudanabaenaceae	++++		++++	
	++++	++++	++++	
Chlorophyta				
Actinastrum sp.	+++	+++	+++	
Coelastrun sp.	++++	+++	++	
Crucigeniella sp.	++	+++	+++	
Desmodesmus sp.1	++++	++++	++++	
Desmodesmus sp.2	++++	+++	+++	
Desmodesmus sp.3			+	
Dictyosphaerium sp.	++	+++	++	
Monoraphidium sp.	++	++++	++++	
Oocystis sp.	+	+++	++	
Pediastrum sp.	+	+		
Scenedesmus sp.1	++++	++++	++++	
Scenedesmus sp.2	++	+++	++++	
Scenedesmus sp.3	+	++		
Tetradriella sp.	+	+++	++++	
Tetraedron sp.	++	+++	+	
Tetrastrum sp.	+		++	
Chlorococcales	++++	++++	++++	
Bacilariophyta				
<i>Cyclotella</i> sp.	++++	++++	++++	
Navicula sp.	++++	+++	++	
Navicula sp. Nitzschia sp.	++++	++++	+++	
Naviculaceae				
	+++	++	+++	
Euglenophyta				
<i>Euglena</i> sp.	+++	++++	++++	
Strobomonas sp.		+		
Trachelomonas sp.	++++	++	+++	
Dinophyta				
Peridiniales	+++	+++	++	
Cryptophyta				
Unidentified taxon	+	++	++	

Table 3 Taxons of phytoplankton observed during the culture of Penaeus vannamei.

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	Treatment			
Taxon	Dolomitic limestone	Algen®	Algen®	
	(1,000 kg/ha)	(250 kg/ha)	(500 kg/ha)	
Rotifera				
Brachionus sp.	++	+++	+++	
Keratella sp.	+	+	++	
<i>Filinia</i> sp.		+++	++	
Lecane sp.	+	+		
Synchaeta sp.	+			
Unidentified taxon	+++	++++	+++	
Crustacea				
Copepod	++++	+++	+++	
Cladocera	+	+	+	
Nauplii	++	++	+	
Protozoa				
Ciliate 1	++	+++	++++	
Ciliate 2	++	+++	++++	
Ciliate 3	++	+	++	
Ciliate 4	+			
Coleps sp.		+		

Table 4 Taxons of zooplankton observed during the culture of *Penaeus vannamei*.

Table 5 Zooplankton density observed during the culture of Penaeus vannamei.

		Treatment			
Taxon	Dolomitic limestone	Algen®	Algen®	Teste t	
	(1,000 kg/ha)	(250 kg/ha)	(500 kg/ha)	р	
Rotifera (Inds./L)	199.0 ± 25.9	959.0 ± 72.8	$2.391.0 \pm 175.3$	0.278	
Crustacea (Inds./L)	110.0 ± 8.6	308.0 ± 23.9	432.0 ± 45.0	0.607	
Protozoa (Inds./L)	31.0 ± 3.7	58.0 ± 2.0	38.0 ± 3.2	0.698	

	Treatment			
Parameter	Dolomitic limestone	Algen®	Algen®	
	(1,000 kg/ha)	(250 kg/ha)	(500 kg/ha)	
Survival (%)	66.3	67.8	63.2	
Initial weight (g)	0.02	0.02	0.02	
Final weight (g)	10.5	10.6	11.7	
DWG (g/dia)	0.106	0.107	0.118	
WWG (g/semana)	0.742	0.749	0.826	
SGR (%/dia)	10.60	10.70	11.80	
Productivity (kg/ha)	4,873	5,030	5,176	
FCR	1.80	1.75	1.70	

Table 6 Zootechnical performance observed during the Penaeus vannamei culture.

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