AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of the larval density and food ration on *Penaeus vannamei* (Boone, 1931) zoea

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ABSTRACT

Objective: To analyze the combined effect of food and larval density in order to determine the combination which the best results of the development, growth and survival of the *Penaeus vannamei* zoea are obtained.

Desing/methodology/approach: The experiment consists of evaluating 16 treatments resulting from the combination of four larval densities: 100, 200, 300 and 400 larvae L^{-1} and four densities of *C. muelleri* as food, adjusting the rations in each of the three phases of the larval development.

Results: The highest values of the development index (3.7), growth in terms of total length (2.86±0.09 mm) and dry (62.13±10.41 μg larvae^{-1}) and organic weight (50.83±7.51 μg larvae^{-1}), as well as the survival (88.30±9.10%) at the end of the experiment were obtained in treatment 3, which consisted of an intermediate larval density (300 larvae L^{-1}) and low food concentration.

Study limitation/implications: For a distintic species of microalga as food, it will be required to evalue the density to use.

Conclusions. In order to optimize the performance of shrimp larval cultivation, the use of a density of 300 larvae L^{-1} and rations of 50 to 80×103 cel mL^{-1} of *C. muelleri* are recommended for future experimental cultivation of *P. vannamei* zoea.

Keywords: *Chaetoceros muelleri*, development, growth, shrimp, survival.

Citation: BermudesLizárraga, J. F., Flores-Alarcón, J. M., Nieves-Soto, M., & López-Peraza, D. J. (2022). Effect of the larval density and food ratio on *Litopenaeus vannamei* (Boone, 1931) zoea. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i8.2255

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: April 24, 2022.
Accepted: July 19, 2022.
Published on-line: August 31, 2022.

Agro Productividad, 15(8). August. 2022. pp: 115-124.

INTRODUCTION

Shrimp is a resource of great importance in Mexico, due to the high demand of the population for this resource, the trend in shrimp farming is the production of high biomass per unit area. However, one of the greatest challenges faced by this activity is the massive production of shrimp larvae and juveniles of and adequate nutritional quality, required by commercial producers (Pérez-Morales *et al.*, 2016). In this sense, the quantity and nutritional quality of the food supplied to the organisms is crucial, since, directly influences the growth, development and survival of the larvae (Martínez-Córdova *et al.*, 2014).

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Various balanced feeds have been formulated for cultured organisms however, live food remains essential, at least in the critical phases of development (larvae and juveniles) of species of commercial interest (Sánchez-Estudillo, 2011). Live food has several advantages over balanced feed, including its high nutritional value, and greater availability and digestibility of nutrients (Castro *et al*., 2003).

Among the live food mostly used by shrimp larvae production, there are different species of microalgae belonging to the genera of *Chaetoceros*, *Skeletonema*, *Tetraselmis*, *Thalassiosira*, *Dunaliella* and *Isochrysis*, as well as zooplankton organisms such as the *Artemia*; the latter is suitable for feeding white shrimp mysis and postlarvae. However, laboratory feeding protocols are commonly based on personal or empirical experiences, which means that the feed supplied is not efficiently used (Aguirre-Hinojosa *et al*., 1999, Piña-Valdez *et al*., 2006). A high density of organisms in the cultivation can cause negative effects on its yield (Mena-Herrera *et al*., 2006; Li *et al*., 2007; Neal *et al*., 2010; Sookyng *et al*., 2011), associated, mainly, to competition for food and aggression between individuals (Sellars *et al*., 2004; Araneda *et al*., 2008; Krummenauer *et al*., 2010). It is essential to establish an adequate relationship between the density of larvae in the cultivation and the food ration to be supplied. In this sense, some studies have focused on the search for the density of postlarvae (Krummenauer *et al*., 2010; Lorenzo *et al*., 2016) and juveniles (Neal *et al*., 2010; Costa *et al*., 2016) of shrimp which a higher yield is obtained under different culture conditions. Efforts have also been made to determine the appropriate concentrations of microalgae (from 30 to 110×10^3 cel L^{-1}) as food for shrimp zoea, in culture densities ranging from 120 to 150 larvae L^{-1} (Kumlu, 1998; Valenzuela-Espinoza *et al*.,1999; Artiles-Rodríguez, 2000; Piña-Valdez *et al*., 2006; Costa *et al*., 2016). The highest survival (>80%) of the *Penaeus schmitti* zoea was obtained when they were fed with the microalgae *Chaetoceros gracislis* at a rate of 30 to 60×10^3 cel L^{-1} (Artiles-Rodríguez, 2000), while in *P. vannamei* (>51% survival) was recorded using the microalgae *C. muelleri* as food, adjusting the rations in each of the three substages of zoea of 100, 150 y 200×10^3 cel L^{-1}, respectively (Piña-Valdez *et al*., 2006).

Nevertheless, there is no standard feeding protocol established according to the stage and density of the larvae that guarantees a better use of the food and therefore a better crop yield. For these reasons, the main objective of this study was to determine the concentration of food (*C. muelleri*) and the density of the larvae which the best development, growth, and survival of the zoea larvae of *L. vannamei* are obtained.

MATERIAL AND METHODS

The *P. vannamei* larvae used in this study were donated by the company "Proveedora de Larvas S.A. de C.V." (FITMAR). The experiment began when 70% of the larvae were at nauplii V and ended on the fifth day, when 90% of the larvae in the treatments were at mysis I. The experiment consisted of evaluating 16 treatments resulting from the combination of four initial larval densities: 100, 200, 300 and 400 larvae L^{-1} and four food rations of *C. mulleri*, adjusting the rations in each of the three phases of development (Table 1). The concentrations added were from 50 to 200×10^3 cel mL^{-1} for zoea I, from 65 to 260×10^3 cel mL^{-1} for zoea II and from 80 to 320×10^3 cel mL^{-1} for zoea III.

Table 1. Treatments (T) resulted from the combination of four densities (D) of larvae and four rations of *Chaetoceros muelleri* microalgae as food (F) for *Penaeus vannamei* zoea.

	Food ration (10^3 cel mL^{-1})			**Density (larvae L^{-1})**			
	Zoea I	**Zoea II**	**Zoea III**	**D1 100**	**D2 200**	**D3 300**	**D4 400**
F1	50	65	80	T1	T2	T3	T4
F2	100	130	160	T5	T6	T7	T8
F3	150	195	240	T9	T10	T11	T12
F4	200	260	320	T13	T14	T15	T16

Each treatment was performed in quadruplicate. In total, 64 circular experimental units with transparent walls of 12 L capacity were used, which were randomly distributed on four wooden shelves of three levels each. The temperature in the treatments was maintained at 30 °C with the help of a 50 W titanium heater (Finnex model HMT50), 35 ups of salinity and continuous aeration. It should be noted that the experiment was repeated twice, therefore, the data presented in this work represent the mean of 8 replicates, four per experiment.

Production of the microalgae *Chaetoceros muelleri*. For the cultivation of the microalgae, seawater filtered to 1 μm and passed through a filter with an activated carbon cartridge was used. The filtered water was stored in 200 L containers where 5% sodium hypochlorite was added at a rate of 1 mL L^{-1} for sterilization at least for least 24 h. At the time of use, residual chlorine was removed by adding sodium thiosulfate at a rate of 60 mg L^{-1}. The F medium (Guillard and Rhyther, 1962) for cultures was used.

The cultures were carried out in transparent polyethylene terephthalate containers of 16 L capacity each one. The cultures were made in series, harvesting daily at 48 h. They were kept with constant aeration and a light intensity of 6000-6500 lux. Cell density was estimated by counting in a compound microscope (Olympus model CH30), using a Neubauer haemocytometer camber.

Analysis of the samples. During the experimental cultures of the larvae, the development index, growth in terms of total length and in dry and organic weight, as well as survival, were estimated.

Larval development index. 30 larvae were taken from each of the experimental units at intervals of 6 h, which were observed directly under a stereoscopic microscope (Leica model Zoom 2000) to identified the development phase. Then, they were immediately returned to their respective treatments, thus minimizing sampling mortality. Subsequently, the larval development index (ID) was determined using the following equation proposed by Villegas and Kanazawa (1979):

$$ID = \frac{\sum in_i}{n}$$

i is the absolute value attributed to each larval stage (nauplii V=0, zoea I=1, zoea II=2, zoea III=3, mysis I=4, mysis II=5, mysis III=6, PL1=7). *ni* is the number of organisms

of the corresponding phase of value *i* found in a sample, and *n* is the total number of specimens observed in each sample.

Growth. The total length of the larvae was measured every 24 h; for this, 30 larvae per treatment were randomly selected and placed in the fixative solution described by Correa Sandoval and Bückle Ramírez (1993). Subsequently, the larvae were measured using a compound microscope (Olympus model CH30), equipped with an eyepiece reticle.

To determine the initial and final dry and organic weight of the larvae (n=300), they were concentrated on Whatman GF/C glass fiber filters of 25 mm diameter previously calibrated at constant weight. After the residual salt was eliminated with 4% ammonium formate, the filters were placed at a temperature of 60°C for drying and weighing. Once the constant dry weight of the samples was obtained, they were incinerated at 250 °C for 4 h, to obtain the ash weight of the larvae. The organic or ash-free weight of the larvae was calculated by the difference between the dry weight and the inorganic or ash-free dry weight of the larvae.

Survival. was determined every 24 h by direct counts of live larvae, in 500 mL samples from each experimental unit. It is worth mentioning that, daily, a 30% seawater exchange was carried out in the experimental units, and based on the estimation of the survival of the larvae, the volume of the treatments was adjusted to keep the initial density of the organisms as a constant, and thus avoid density-dependent responses.

Statistical analysis. After verifying the normality (Lilliefors test) and the homogeneity of variance (Bartlett test) to the data: development index, total length, and survival of the shrimp zoea, a two-way analysis of variance (ANOVA) was applied to determine the significant differences between treatments. When the ANOVA revealed significant differences, Tukey's multiple comparison test was applied to identify these differences (Figures 1, 2, 3 and 5). The results are expressed as mean ± standard deviation (n=8). Symbol * and different letters indicate significant differences between treatments. All tests were performed with a significance level of (α) of 0.05 (Zar, 2010).

RESULTS AND DISCUSSION

Development index. The development index of shrimp larvae showed a similar trend until 90 h (Figure 1), where a total average of 2.98±0.03 was recorded, which indicated that 98±0.03% of the larvae in the experiment were at the zoea III stage. The highest values (3.97) of the DI at the end of the experiment were recorded at T1 and T3, but they only showed significant differences with the values recorded at T9 and T13 (Figure 1). The maximum DI obtained was higher than the maximum value (DI=3.17) recorded by Piña-Valdez *et al.* (2005) feeding with the same microalgae (*C. muelleri*), and same period of time. But the studies carried out by Medina-Jasso *et al.* (2004) and Bermudes-Lizárraga (2009) recorded an accelerated development of *L. vannamei* zoea feeding monogal diets of *C. muelleri* and *Thalssiosira weissflogii*, respectively. These authors recorded similar values of DI (above 95% of larvae in mysis I) in a shorter period of time; at 72 and 102 h, that is, up to 48 h earlier than in the present study. Although Medina-Jasso *et al.* (2004) recorded an advance in DI, the total length of the larvae was similar to that obtained in this study.

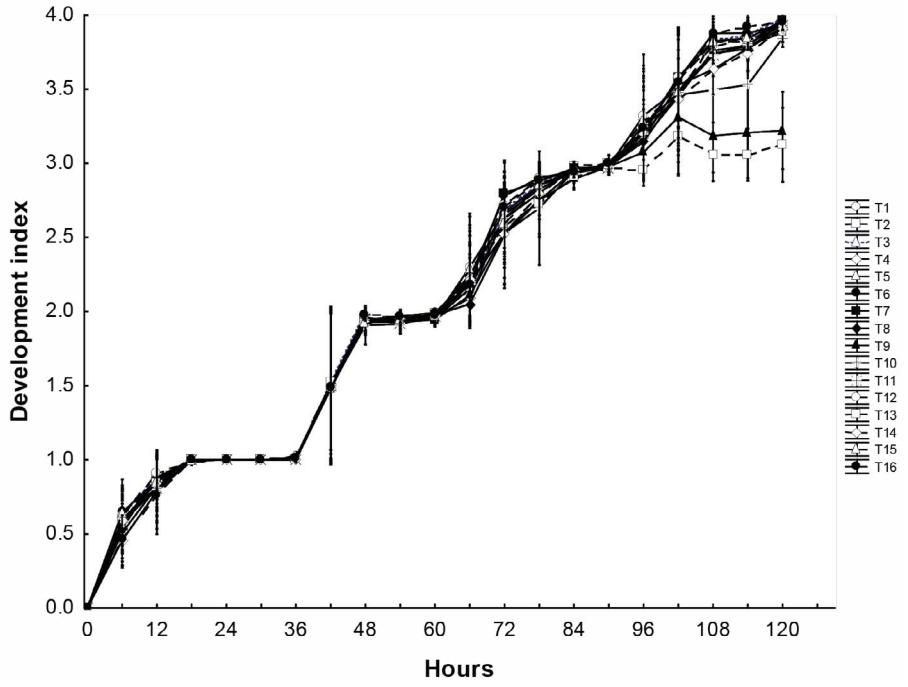

Figure 1. Development index of *P. vannamei* zoea fed with *C. muelleri* microalgae.

Likewise, the DI registered at 120 h was similar to that registered in zoea of *L. stilyrostris* fed with a mixture of *C. calcitrans*, *T. suecica* and *Artemia nauplii* (Isiordia-Pérez and Puello-Cruz, 2007). This suggests that the zoea of *L. stylirostris*, like those of *P. vannamei*, could be fed only with phytoplankton (Kumlu et al., 1998; Valenzuela-Espinoza, 1999; Piña *et al.*, 2005, 2006; Bermudes-Liárraga, 2009), with not need to add zooplankton organisms such as *Artemia*, which would imply a reduction in feeding costs.

Growth. There were no significant differences between the total length of the larvae of the treatments until day 3, however, at day 4, T13 was significantly lower than the rest of the treatments, which did not present differences among them (Figure 2). At day 5, the highest average of the total length recorded was 2.86±0.09 mm in T3, which represented an increase by 6.6 times compared to the initial value of the experiment (0.43 mm), however, this result was only significantly different from the values presented at T9 (2.42±0.30 mm) and T13 (2.40±0.14 mm).

The average unit dry weight at the beginning of the experiment was 2.59±0.20 μg larvae^{-1}, the organic weight was 2.31±0.05 μg larvae^{-1} and the percentage of organic matter with respect to dry weight (OW/DW) was 89.55±5.25%. The highest unit dry weight at the end of the experiment was 62.13±10.41 μg larvae^{-1} in T3, which indicates that the larvae increased their size about 24 times, compared to the initial value. This unit dry weight value was statistically different only from what was recorded at T9 and T13, which did not present differences among them (Figure 3). Similarly, T3 recorded the highest unit organic weight (50.83±7.51 μg larvae^{-1}) at the end of the experiment (22 times more in relation to the initial value), being significantly different from the values recorded in treatments 1, 5, 9, 10, 13 and 14. Likewise, the maximum unit dry weight obtained in this work is almost double that recorded by Piña-Valdez *et al.* (2005).

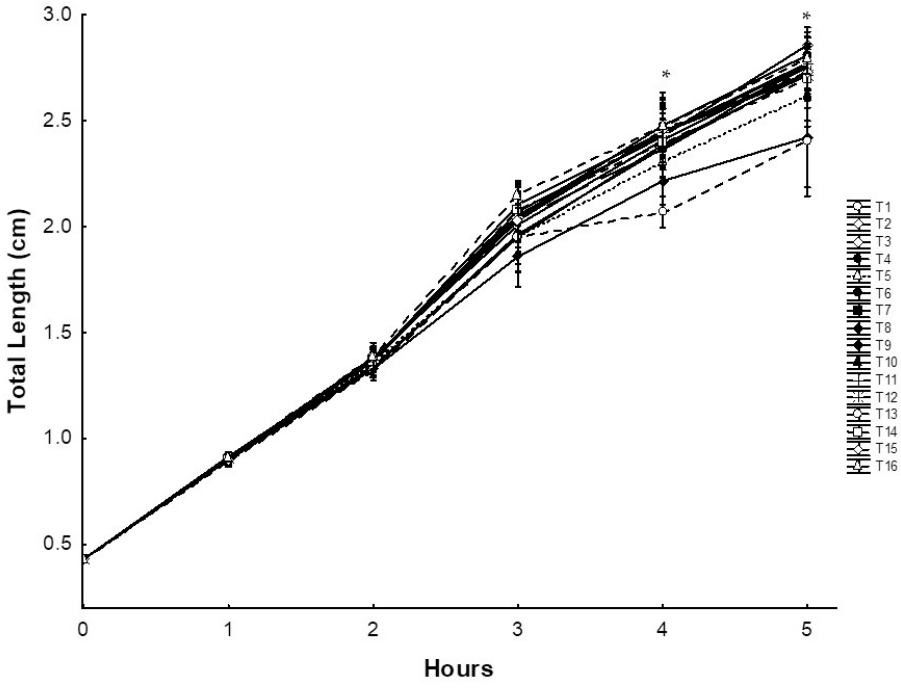

Figure 2. Total length (mm) average *P. vannamei* zoea fed with *C. muelleri* microalgae.

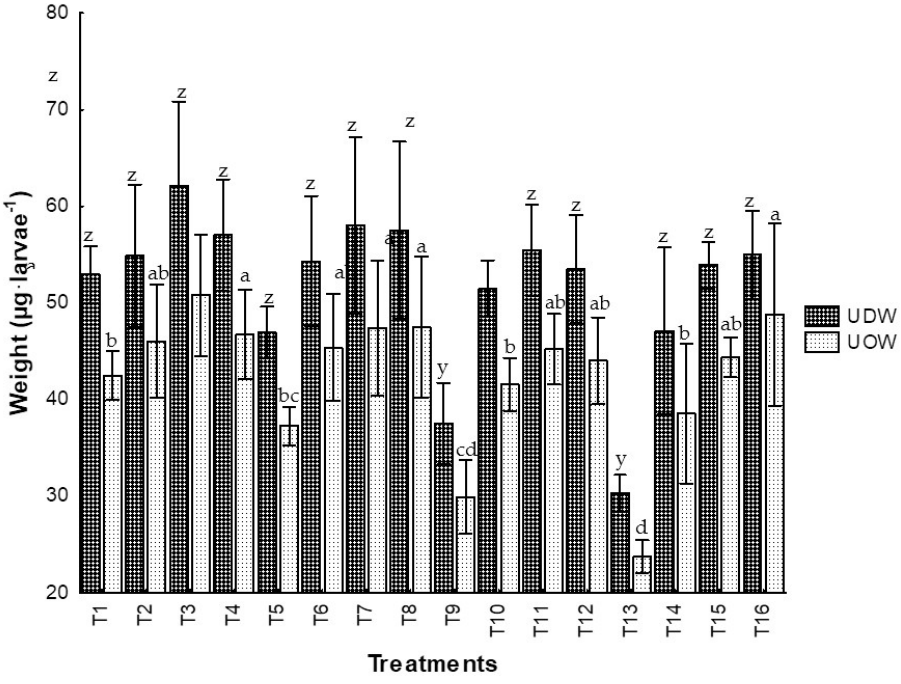

Figure 3. Final unit dry weight (UDW) and organic weight (UOW) of *P. vannamei* larvae fed with *C. muelleri* microalgae.

In relation to the final OW/DW content, the values fluctuated between 78.45±2.80 and 84.04±4.52% (T13 and T2, respectively), without significant differences between treatments (Figure 4).

Survival. In the same way as in the total length, the differences between the percentage of survival among the treatments were recorded from the fourth day of culture (Figure 5). On this day, T3 recorded the highest survival (88.30±9.10%), however, it only presented significant differences with T1, T4, T5, T7 and T11, which were not differences among them. At the end of the experiment, T3 registered a final survival of 87.67±7.11%, being significantly higher than the rest of the treatments. The maximum final survival of this study was higher than the values recorded in previous studies (Medina-Jasso *et al.*, 2004; Piña-Valdez *et al.*, 2005, 2006; Isiordia-Pérez *et al.*, 2007) for *P. vannamei* zoea, but it was similar to those obtained for *P. indicus* fed with mixed diets of *Tetraselmis chuii* and *Skeletonema costatum* (Kumlu, 1998), *L. vannamei* fed with monoalgal diets of *C. muelleri* (Valenzuela-Espinoza *et al.*, 1999), *L. schmitti* fed with *C. gracilis* (Godínez *et al.*, 2005) and for *L. stylirrostris* fed with *C. calcitrans* (Godínez *et al.*, 2005), which survival values from 87 to 92.7% were recorded.

As in other studies (Kumlu, 1998; Bermudes-Lizárraga, 2009), the increase in the amount of food supplied to the *P. vannamei* zoea did not reflect a proportional increase in growth in terms of total length and dry and organic weight, nor in the DI. On the contrary, treatments 9 and 13, which had a low larval density (100 larvae L^{-1}) and a high amount of food, particularly T13 (D100C4), were the ones that registered the lower values of these

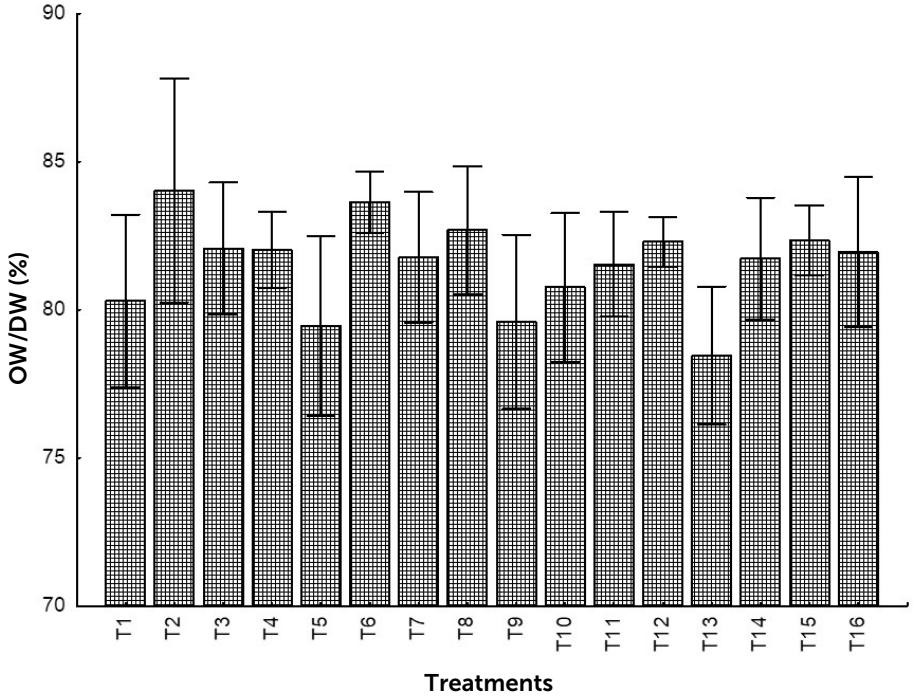

Figure 4. Percentage of organic matter with respect to dry weight (OW/DW) *L. vannamei* shrimp larvae fed with *C. muelleri* microalgae. See Table 1 for the description of the treatments. Mean ± standard deviation (n=8). There were no significant differences between treatments ($p<0.05$, two-way ANOVA).

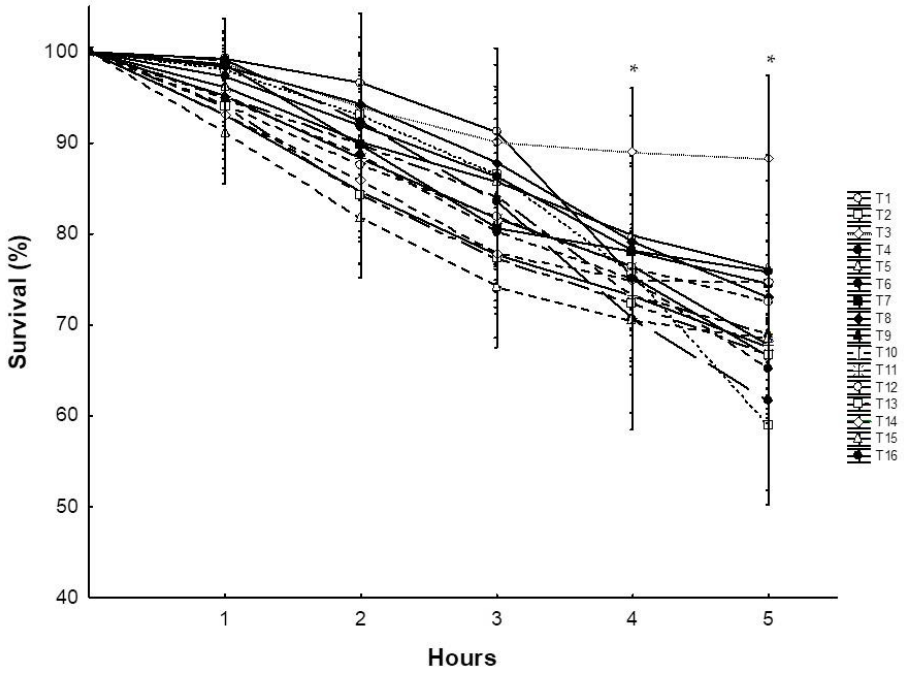

Figure 5. Survival (%) of *P. vannamei* larvae fed with *C. muelleri* microalgae.

variables. These results suggest that the larvae in these treatments were probably overfed, which could have caused an increase in toxic metabolites resulting from the metabolism of the microalgae, coupled with the decomposition of the dead matter on the bottom (Artiles-Rodríguez, 2000; Godínez *et al.*, 2005), directly affecting the optimal performance of the larvae. However, survival did not reflect a negative effect of this possible overfeeding.

Unlike previous studies with shrimp postlarvae (Otoshi *et al.*, 2006; Krummenauer *et al.*, 2011; Lorenzo *et al.*, 2016) have revealed that high densities of organisms in cultures (≥300 postlarvae L^{-1}) affected their performance; in the present study, no clear effect of the density of *P. vannamei* zoea on the estimated variables was observed. Even, contrary to what was expected, T4, which was the result of the combination of the highest density of larvae (D4=400 larvae L^{-1}) and the lowest concentration of food (C1), registered similar values to the treatments with the lowest larval density. This suggests two situations: the first is that, at least during the zoea stage, it is possible to maintain cultures at high densities (400 larvae L^{-1}), and the second is that the food provided was sufficient to meet the nutritional needs of the larvae under the culture conditions used.

In experimental cultures of juveniles of *P. vannamei* juvenile (Lin and Chen, 2001) and *L. schimiti* (Barbieri, 2010), there is evidence that high mortalities are associated with high shrimp densities (≥100 juveniles m^{-2}) mainly due to the high concentrations of ammonium that can be recorded in the crops. In addition to the possibility of the presence of cannibalism, associated with stress factors due to density and lack of food (Arnold *et al.*, 2005). However, in the juveniles of *P. vannamaei* cultivated in Biofloc systems, the results have been encouraging, since there has been no negative effect on the culture yield as the density of the organisms increases at densities of up to 364 juveniles m^{-2} (Neal *et al.*,

2010), as long as nutritional requirements are met and intense light or natural light is provided (Costa *et al.*, 2016). It is worth mentioning that, although the evidence of the aforementioned works does not reveal, in a statistical way, a negative effect on the survival and growth of shrimp as a result of the high densities of the cultivation, quantitatively, the highest yields of the culture were obtained at the intermediate or low densities.

CONCLUSION

The best results in relation to growth, development and survival were obtained in T3 (D300C1), whose larval density was intermediate (300 larvae L^{-1}) and the concentration of food was low. Therefore, the use of these conditions is recommended for subsequent cultures of *P. vannamei* zoea, as a way of preventing the effects associated with high densities (cannibalism, food shortages, excess of nitrogenous products) and high food concentrations (increase in production costs, excess of toxic metabolites resulting from the metabolism of microalgae, decomposition of organic matter).

ACKNOWLEDGEMENTS

This research was supported by grant PROFAPI2014/111.

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