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# The effects of different feeding rates and re-feeding of *Litopenaeus vannamei* in a biofloc culture system

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ABSTRACT

The natural productivity in biofloc culture systems could be an important source of supplementary food to shrimp, representing savings in artificial feed. The aim of the present study was to evaluate the effects of using different feeding rates for a period of 21 days with a posterior re-feeding period in a microcosm system in the presence of bioflocs. *Litopenaeus vannamei* juveniles (1.14 ± 0.38 g) were stocked at 400 shrimp $m^{-3}$ in 150-L tanks in a biofloc recirculation system in two phases. The feeding rates were calculated considering an expected weekly growth of 1 g $week^{-1}$ and an estimated weekly mortality of 0.5%; each treatment corresponded to a different feeding rate, and each feeding rate corresponded to a fixed food conversion ratio. The first phase (food restriction) lasted 21 days, and the following treatments were used: T0 (no artificial feed addition), T0.3, T0.6, T0.9, T1.2, T1.5, T1.8 and T2.1. In the second phase (re-feeding), the feeding rate was calculated based on the average of the best results in the first phase of the experiment (FCR = 1.45). The re-feeding period lasted for more 29 days. There were no observed significant differences in the water quality parameters among the treatments ($P > 0.05$). At the end of the food restriction, the shrimp in T0, T0.3 and T0.6 presented lower final weights ($P < 0.05$), and the weights in the other treatments did not significantly differ ($P > 0.05$). The survival rate was lower only in T0 in the two phases of the study. The other treatments presented survival rates higher than 95%, with no significant differences among them. The feed intake did not increase during the re-feeding period, indicating that hyperphagia did not occur after a period of food restriction. The SGRs were higher for treatments that received lower amounts of feed in the first phase, and treatments T0, T0.3 and T0.6 presented partial weight compensation compared with the treatments with higher feeding rates. This study indicates that shrimp can be reared in a biofloc system with lower feeding rates, obtaining partial weight compensation and high survival rates and saving up to 24.79% of the artificial feed.



## 1. Introduction

Shrimp is an important commodity in the world seafood market, and virtually all production of *Litopenaeus vannamei* is obtained through aquaculture, with continuing projected growth in the next decade (FAO, 2016). With a well-established production and market, there are issues and challenges in shrimp aquaculture that deserve special attention to improve production and reduce risks. Disease outbreaks, quality and availability of seed stock, environmental management, feed quality and availability and production costs (mainly with feed/fishmeal) are some of the points affecting shrimp production worldwide (Jory, 2016). Biofloc technology (BFT) has been considered an important alternative to manage these limiting factors (Crab et al., 2012). The bioflocs contribute to the water quality of the culture, enabling a more biosecure system and avoiding high water exchange rates (Ray et al., 2011). The aggregates also could improve the immune response of the shrimp emerging as an alternative to grow animals resistant to diseases (Kim et al., 2014). Furthermore, the composition and nutritional value of the bioflocs could contribute to shrimp nutrition, reducing the need for artificial food (Wasielesky et al., 2006; Emerenciano et al., 2012). The sum of all these factors, considering a system in which proper management is applied, can lead to higher yields by reducing the risks and costs associated with diseases, food, and water quality management (De Schryver et al., 2008; Crab et al., 2012; Krummenauer et al., 2014).

The natural productivity promoted in culture systems contains nutritional components considered as growth enhancers (Otoshi

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et al., 2011). Ju et al. (2008) and Martins et al. (2016) reported this "growth-factor" in studies investigating the contribution of natural productivity (primarily diatoms) to the nutrition of shrimp, showing increased growth of the animals. Additionally, Otoshi et al. (2011) also demonstrated that the natural productivity in pond water is a ubiquitous food source, and animals did not have to spend much energy on feeding, using these reserves for rapid growth. In recent studies with biofloc technology systems, management techniques have been used in artificial feeds and in the culture environment to reduce the protein content in aquafeeds (Wasielesky et al., 2006; Xu et al., 2012), evaluate the use of alternative ingredients (plant-based, floc meal) (Ray et al., 2010; Bauer et al., 2012), analyze different carbon sources that have been used to promote biofloc growth (Crab et al., 2010), and investigate the digestive enzyme activity (Xu and Pan, 2012). The results maximized the utilization of the microbial community in tanks as supplemental feed for the reared animals. However, few studies have examined feeding management by observing the response of shrimp to total or partial food restriction and re-feeding periods, or characterized potential compensatory growth responses by evaluating feeding parameters in biofloc culture systems.

Compensatory growth is defined as faster growth during recovery from total or partial food deprivation to achieve the same weight as animals that did not experience any food deprivation (Ali et al., 2003). Compensatory growth could also be observed when animals were subjected to any unfavorable factor, such as high stocking density (Fóes et al. 2016), hypoxia (Wei et al., 2008), or different temperatures (Wu and Dong, 2002); could be observed in different life stages of the organisms (Foss et al., 2009). Recent studies have shown that shrimp biofloc cultures could contribute to this growth acceleration, reflecting the nutritional composition of the aggregates and improved conditions of the animals after being faced with unfavorable culture conditions (Wasielesky et al., 2013; Hostins et al., 2015). Nevertheless, although microbial aggregates could be used as supplemental feed in shrimp nutrition, there are no studies examining whether the total quantity of the feed offered could be reduced or whether the animals could recover sufficient weight after a period of food restriction in this system. In addition, the use of different feeding rates could provide information concerning the need for artificial food during different phases of the growth cycle. This information could further refine the required quantities of food during rearing to improve feed efficiency. Furthermore, feeding restriction, as a trigger for compensatory growth, might be considered an alternative viable strategy for minimizing waste and production costs (Stumpf and Greco, 2015; Zhu et al., 2016).

In this context, the objective of the present study was to evaluate the effects of re-feeding after a period of food restriction (21 days) in which *Litopenaeus vannamei* juveniles were subjected to different feeding rates in a biofloc culture system.

## 2. Materials and methods

### 2.1. Location

The present study was conducted at the Marine Station of Aquaculture Institute of Oceanography, Federal University of Rio Grande in Southern Brazil.

### 2.2. Experimental design and feeding rates

*Litopenaeus vannamei* juveniles were stocked with an initial weight of 1.14 g (±0.38) at an initial stocking density of 400 shrimp/m$^3$ (n = 60). The study lasted 50 days.

**Table 1**
– Feeding rates in different treatments in the two phases of the study.

| Treatment | Phase 1 (21d) | % Feed | Phase 2 (29d) | % Feed |
|---|---|---|---|---|
| T0 | No feed + biofloc | 0 | Re-fed + biofloc | 65.25 |
| T0.3 | Feed + biofloc | 14.29 | Re-fed + biofloc | 65.25 |
| T0.6 | Feed + biofloc | 28.66 | Re-fed + biofloc | 65.25 |
| T0.9 | Feed + biofloc | 42.86 | Re-fed + biofloc | 65.25 |
| T1.2 | Feed + biofloc | 57.14 | Re-fed + biofloc | 65.25 |
| T1.5 | Feed + biofloc | 71.43 | Re-fed + biofloc | 65.25 |
| T1.8 | Feed + biofloc | 85.71 | Re-fed + biofloc | 65.25 |
| T2.1 | Feed + biofloc | 100 | Re-fed + biofloc | 65.25 |

In the first phase, T0 did not received any artificial feed. The percentages of feed (% Feed) were calculated assuming that T2.1 was the 100% and the calculation followed the methodology proposed by Garza de Yta et al. (2004), assuming different FCR's and weekly growth rate of 1 g per week.

The study was divided into two phases: (1) Food restriction (21 days) – Eight treatments corresponding to eight different feeding rates (in triplicate), which were: T0 (no food addition), T0.3, T0.6, T0.9, T1.2, T1.5, T1.8 and, T2.1; and (2) Re-feeding (29 days) – All treatments were fed with the same amount of artificial feed, calculated based on the mean FCR of the better performances obtained in the Phase 1 (1.45). Table 1 summarizes the different treatments and the quantities of feed offered in two phases.

The daily feed was calculated based on the methodology of Garza de Yta et al. (2004). The feeding rates were calculated, assuming different FCR's and 1 g of weight gain per week. Each assumed FCR value corresponded to a different feeding rate. The following formula was used to calculate daily feeding:

$$\text{Feed (daily)} = (\text{Number of Shrimp} \times \text{Expected Weekly Growth Rate}$$
$$\text{x Expected FCR})/7.$$

The feed was offered twice a day via feeding trays. Uneaten feed was removed from the feed trays every morning and dried in an oven at 60 °C until constant weight. Subsequently, the final dry weight was recorded using a digital balance with 0.01 g of precision. The shrimp were fed a 38% protein commercial shrimp diet (Poti Active 38, 1.6 mm, D'Aguabi, Guabi Nutrição e Saúde Animal S.A., Campinas, São Paulo, Brazil). Feed leaching of the artificial feed were measured prior to initiating the study to calculate the food conversion ratio and feed intake, leaching of 24 h was 66.84%. The diet was composed by a maximum of 10% of moisture, minimum of 38% of crude protein, 7.5% of ether extract, 5% of crude fiber, 13% of mineral matter, 3% of calcium and 1.45% of phosphorus (information provided by the manufacturer).

Table 1 To isolate the potential negative effects of different feeding rates on water quality, a water biofloc recirculation system was designed. This system was proposed to observe only the effects of different feeding rates and the contribution of the bioflocs on shrimp performance. The system contained twenty-four 150-L tanks (microcosm tanks) with a bottom area of 0.5 m$^2$ and individual aeration. All tanks had gravity-driven water output into a return pipe leading to a matrix tank with a 20-m$^3$. In this matrix tank, an inoculum of 10% of old biofloc was used to promote natural productivity prior to the start of the experiment (10 days prior to the storage of shrimp). The water was pumped back into the microcosm tanks using a pump, with a flow rate of 2.08 L/min per tank, totaling 20 water exchanges per day. The water quality in matrix and microcosm tanks was monitored prior to initiating the study to observe whether the concentrations of the main factors maintained the same values in these units. The biofloc was composed by bacteria (isolated and forming aggregates), protozoans (ciliates and flagellates), rotifers and nematodes.