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DIFFERENT DENSITIES IN WHITELEG SHRIMP CULTURE USING BIOFLOCS AND WELL WATER IN SUBTROPICAL CLIMATE

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ABSTRACT

In the last years, there is a strong tendency to produce marine shrimp in biofloc technology. This system promotes the increase of stocking densities using smaller areas. In addition, the use of well water can also be an alternative for farms off the coast. In subtropical areas, the temperature is a very important parameter to be considered, because it can reduce the culture period in ponds. This study aimed to evaluate the feasibility of production of *Litopenaeus vannamei*, comparing two stocking densities (100 and 150 shrimp m^{-2}), use biofloc technology and well water. The experiment was carried out from January to April 2011 (105 days) at the Marine Station of Aquaculture (EMA/FURG), located at Rio Grande city (RS), Southern Brazil. Three replicates ponds (600 m^{2} area each one) were used for both stocking density (post larvae 0.08 ± 0.03 g - PL 40). The water quality parameters and ionic composition of well water were within acceptable range for the *L. vannamei.* In the shrimp performance, the low FCR in both treatments can be attributed to the consumption of microbial flocs by the shrimp. The stocking densities showed statistical differences in productivity: 9,748 and 13,860 kg ha^{-1} for the treatments 100 and 150 shrimp m^{-2}, respectively. Although the water temperature decreased in the last two weeks (19.4 °C), the survival was 97% (100 shrimp m^{-2}) and 88% (150 shrimp m^{-2}). In southern Brazil, the stocking densities 100 and 150 shrimp m^{-2} were suitable showing efficient values of growth, survival and feed conversion of shrimps. However, the density of 150 shrimp m^{-2} was more profitable due to its higher productivity. In this study, ionic ratio was lower than the values of seawater, but it did not affect the shrimp performance. With respect to location, it should be taken into account that climatic conditions in this area allow shrimp culture in ponds during the warmest months.

Key words: *Litopenaeus vannamei*; intensification; temperature.

DIFERENTES DENSIDADES EM CULTIVO DO CAMARÃO BRANCO USANDO BIOFLOCOS E ÁGUA DE SUBSOLO EM CLIMA SUBTROPICAL

RESUMO

Nos últimos anos, há uma forte tendência para produção de camarões marinhos em sistema de bioflocos. Este sistema favorece o aumento das densidades de estocagem utilizando menores áreas. Além disso, o uso de água de subsolo também pode ser uma alternativa para empreendimentos afastados do litoral. Em áreas subtropicais a temperatura é um importante parâmetro a ser considerado, pois pode reduzir o período de cultivo em viveiros. Este estudo teve como objetivo avaliar a viabilidade da produção de *Litopenaeus vannamei,* comparando duas densidades de estocagem (100 e 150 camarões m^{-2}), utilizando bioflocos e água do subsolo. O experimento foi realizado entre janeiro a abril de 2011 (105 dias) na Estação Marinha de Aquacultura (EMA/FURG), localizada na cidade do Rio Grande, RS, Brasil. Os camarões foram estocados em viveiros de 600 m^{2} com pós-larvas de 0,08 ± 0,03 g (PL 40), sendo utilizadas três réplicas para cada tratamento. Os parâmetros de qualidade da água e composição iônica estavam dentro dos limites aceitáveis para *L. vannamei*. No desempenho zootécnico, a conversão alimentar em ambos os tratamentos pode ser atribuída pelo consumo de flocos microbianos pelos camarões. As densidades apresentaram diferença significativa na produtividade: 9.748 e 13.860 kg ha^{-1} para 100 e 150 camarões m^{-2}, respectivamente. Embora a temperatura da água tenha diminuído nas duas últimas semanas (19,4 °C), a sobrevivência foi 97% (100 camarões m^{-2}) e 88% (150 camarões m^{-2}). No sul do Brasil, as densidades testadas 100 e 150 camarões m^{-2} foram adequadas apresentando valores eficientes de crescimento, sobrevivência e conversão alimentar dos camarões. No entanto, a densidade de 150 camarões m^{-2} foi mais rentável devido à alta produtividade. Neste estudo, a relação iônica foi mais baixa do que os valores da água do mar, mas isto não afetou o desempenho zootécnico dos animais. Em relação à localização, deve-se levar em consideração que as condições climáticas nesta área possibilitam cultivo de camarão em viveiros durante os meses mais quentes.

Palavras-chave: *Litopenaeus vannamei*; intensificação; temperatura.

INTRODUCTION

The Northeast region of Brazil is the largest producer of marine shrimp *Litopenaeus vannamei* species, with more than 95% of national production and the domestic market consumes the majority part of this commodity (ROCHA and MENDONÇA, 2015). Most of the farms use semi-intensive systems, with densities between 10 to 30 shrimp m^{-2} and productivity around 2.5 t ha^{-1} per cycle (PONTES, 2006). However, shrimp farming is currently growing in high latitudes, such as in southern Brazil (FREITAS *et al*., 2011).

In Brazilian's subtropical areas, the temperature is a very important parameter to be considered, because it can reduce the culture period in ponds. The temperature influences the oxygen consumption, the growth, frequency of molts and feed conversion (PEREZ-VELAZQUEZ *et al*., 2012). SOARES *et al*. (2012) verified that the shrimps growth rate declined significantly at low temperatures. This occurs because the animals enter in the state of lethargy, confirmed by lower metabolic rates (WALKER *et al*., 2011). Due to this, the grow-out phase in Southern Brazil can vary from six to eight months (PEIXOTO *et al*., 2003).

In the last years, there is a great tendency to produce marine shrimp in biofloc technology (BFT system), in subtropical regions mainly because use of nurseries in greenhouses (VENERO *et al*., 2009; BROWDY *et al*., 2017). Also, this system promotes the increase of stocking densities using smaller areas (SAMOCHA *et al*., 2012), maintenance of water quality and protection against diseases (KIM *et al*., 2014). Currently, white spot outbreaks are causing damage in the Northeast of Brazil and one of the alternatives found by farmers is apply BFT system. Studies show that the immunostimulating action of the biofloc contributes to the disease control (AALIMAHMOUDI *et al*., 2017).

The bioflocs formation is based on the addition of carbon in the pond water. The ratio carbon: nitrogen (C:N) is modified and provides the growth of heterotrophic bacteria that convert inorganic nitrogen in microbial protein (AVNIMELECH, 1999). The BFT system using high stocking densities is beginning commercially in Brazil, because the deployment method costs more than traditional systems, due to the construction of physical structures and equipment acquisition (POERSCH *et al*., 2012; WASIELESKY *et al*., 2013b). However, the increase in the cost can be offset by the higher productivity achieved in this system (BOYD and CLAY, 2002; REGO *et al*., 2017).

Although researches indicate that the use of high stocking densities in traditional systems can result in cannibalism and competition (OTOSHI *et al*., 2007), the culture of *L. vannamei* in BFT system usually shows high survival. For instance, in Southern Brazil, KRUMMENAUER *et al*. (2011) compared stocking densities (150, 300 and 450 shrimp m^{-2}) in raceways, and the results of shrimp performance were better at 300 shrimp m^{-2}, as the study of LIU *et al*. (2017). In ponds, FRÓES *et al*. (2012) and BURFORD *et al*. (2004) tested the densities of 85 and 120 m^{-2} shrimp, respectively, and reached survival above 90%. Furthermore, HARGREAVES (2013) suggested densities between 125 to 150 shrimp m^{-2} to improve the stabilization of the microbial community.

Marine shrimp can be cultured in low salinity water, if it has a similar ionic profile of seawater (DAVIS *et al*., 2004). In Latin America, there is little information about shrimp culture using well water. However, countries like the USA and China apply this concept for decades (ROY *et al*., 2010). Informally, it is known that in the Northeast region of Brazil some farms operate with well water and low salinity too (NUNES and ROCHA, 2015). Thus, this study aimed to evaluate the feasibility of production of *L. vannamei,* comparing two stocking densities (100 and 150 shrimp m^{-2}), use biofloc technology and well water (BFT) in ponds in subtropical areas (extreme south of Brazil).

METHODS

The experiment was carried out from January to April 2011 (105 days) at the Marine Station of Aquaculture (EMA), Federal University of Rio Grande (FURG), located at Rio Grande city (32°12'16" S; 52°10'41" W), Rio Grande do Sul state, Southern Brazil. The experimental design consisted of two treatments of 100 and 150 shrimp m^{-2}, with three replicates each. The outdoor ponds had 600 m^2 area each one and lined with high density polyethylene (HDPE). The ponds were filled with well water located 20 meters away from experiment at nine meters depth. The well water was pumping by a centrifugal Jacuzzi pump – 7.5 HP (model 75 JM3-T). The bioflocs inoculated in the ponds (1000 L inoculum per pound) were taken from tank in a greenhouse with a stabilized nitrification process. The aeration was maintained 24 hours a day by using paddle wheel aerators (Trevisan® - 17 hp ha^{-1}) in the ponds. After filling the grow-out ponds with well water and bioflocs inoculation, the post larvae of *L. vannamei* with initial weight of 0.08 ± 0.03 g (PL 40) were transferred from the larviculture to the ponds as defined in the treatments.

Initially, the shrimps were fed three times a day with a species-specific commercial feed (Potimar 40 J) containing 40% crude protein. After reach a weight of 1.0 g, we used commercial feed (Potimar 40 J) containing 38% crude protein. The daily feeding rate was 60% of shrimp biomass. Posteriorly, the tables contained in JORY *et al*. (2001) were followed until the end of the experiment. Weekly, fifty shrimps were randomly sampled from each pond and individually weighed to determine the growth and adjust the amount of feed to be supplied to the animals.

The well water samples were analyzed using a photo colorimeter (WTW Photolab S6) and specific assay kits (sodium, calcium, magnesium and potassium) at Federal University of Santa Maria, Brazil. The proportion of major ions of well water (17 ppt) were: Na^+: K^+ (20: 1), Ca^{2+}: K^+ (0.7: 1), Mg^{2+}: Ca^{2+} (0.19: 1), Mg^{2+}: K^+ (0.13: 1) and seawater (35 ppt) (GOLDBERG, 1963): Na^+: K^+ (28: 1), Ca^{2+}: K^+ (1.08: 1), Mg^{2+}: Ca^{2+} (3.4: 1), Mg^{2+}: K^+ (3.6: 1).

The dissolved oxygen (DO), temperature and pH were monitored twice a day and salinity three times a week with multi-parameter equipment (YSI® Model 556). Alkalinity (APHA, 1998) and TA-N (UNESCO, 1983) were analyzed every three days. Weekly, nitrite (NO_2^--N), nitrate (NO_3^--N) orthophosphate (PO_4^{3-}-P) and the total suspended solids concentration (TSS) were analyzed following a methodology adapted from STRICKLAND and PARSONS (1972). The average values of water quality and shrimp performance parameters were subjected to normality and homogeneity and analyzed using Test "t" student. We used the software STATISTICA 7.0® (StatSoft Inc. 2004, Tulsa, Oklahoma, USA) for the statistical analysis.

RESULTS

The physical-chemical parameters of water culture during experimental period (Table 1) did not differ significantly among treatments ($p>0.05$).

DISCUSSION

The values of dissolved oxygen, pH and alkalinity of the water remained within the acceptable range for the *L. vannamei* during the experiment (VAN WYK and SCARPA, 1999). The total suspended solids mean values remained according to the recommended level for the BFT system, less than 500 mg L^{-1} (GAONA *et al*., 2011). Although the temperature not reached the ideal for shrimp growth (28 to 32 °C) (VAN WYK and SCARPA, 1999), but in this work the mean value of temperature (24 °C) probably did not reduce survival.

The stocking density is one of the factors that determine growth, survival and productivity (RUIZ-VELAZCO *et al*., 2010). Several authors report an inverse relationship between this factor and shrimp performance (FÓES *et al*., 2011; KRUMMENAUER *et al*., 2011; WASIELESKY JUNIOR *et al*., 2013a). The densities of 100 and 150 shrimp m^{-2} tested in this study did not present this relationship, since the highest density showed highest productivity resulting in a significant difference. This also means that these densities are suitable in the culture. Although the BFT System supports higher densities than presented here, until the moment, these densities were the highest tested in ponds in this region.

The highest productivity (13.8 ton ha^{-1}) was similar to the study of McINTOSH (1999), at densities of 115 to 125 shrimp m^{-2} and productivity of 13.4 ton ha^{-1} in lined ponds with area of 1.6 ha. Also in BFT system operating with well water and low salinity (2.0 ppt), DAVIS *et al*. (2004) reached productivity of 12 ton ha^{-1} with densities of 109 shrimp m^{-2} in earthen ponds of 0.1 ha.

The low apparent feed conversion rate (FCR) in both treatments can be attributed to the consumption of microbial flocs by the shrimp (WASIELESKY JUNIOR *et al*., 2006a). This conversion was lower than the traditional crops. GUNALAN *et al*. (2011) found FCR value of 1.35 for *L. vannamei* cultured at densities of 50 to 61 shrimp m^{-2} in ponds of 0.9 ha and survival of 80%. TAW *et al*. (2008) using stocking densities between 145 and 280 shrimp m^{-2}, obtained FCR values of 1.2 and 1.1. However, these authors applied partial harvests, unlike this study.

In contrast to ARANEDA *et al*. (2008), they tested densities of 90, 130 and 180 shrimp m^{-2} with recirculation system and well water and achieved the WGR values of 0.38, 0.34 and 0.33 g, respectively. The period of culture was double (210 days) of this study. The average final weight was 11.72 g, while for these densities were 10.05 g (100 shrimp m^{-2}) and 10.5 g (150 shrimp m^{-2}), respectively. The authors confirm that these values are inferior when compared to the intensive system. Furthermore, water temperature remained below 26 °C, but differences in weight gain were attributed to the stocking densities.

Besides the stocking density, the temperature has an influence on physiological processes and consequently in the growth rate of the shrimp (GUO *et al*., 2010). The temperature is related to ion transport, in which the high temperatures provide better osmoregulation (GILLES and PEQUEUX, 1983). As previously mentioned, this physical parameter may have contributed to the low values of WGR (0.67 to 100 shrimp m^{-2} and 0.7 to 150 shrimp m^{-2}) because the culture period extended until the autumn. The water

Table 1. Mean values (±SD) of physicochemical parameters of water culture for the treatments: different stocking densities (100 and 150 shrimp m^{-2}) for *L. vannamei* shrimp.

Parameters	**Treatment 100 shrimp m^{-2}**	**Treatment 150 shrimp m^{-2}**
Dissolved oxygen (mg L^{-1})	8.34 ± 2.57	8.09 ± 2.48
Temperature (°C)	24.19 ± 1.92	24.17 ± 1.88
pH	8.02 ± 0.29	8.09 ± 0.25
Alkalinity (mg $CaCO_3$ L^{-1})	178.45 ± 15.56	200.68 ± 23.72
Salinity	20.80 ± 1.36	20.83 ± 1.39
TSS (mg L^{-1})	401.24 ± 197	483.32 ± 289
TA-N (mg L^{-1})	0.58 ± 0.57	0.50 ± 0.70
N-NO_2^- (mg L^{-1})	0.58 ± 0.77	1.36 ± 1.60
N-NO_3^- (mg L^{-1})	1.80 ± 1.24	1.66 ± 0.91
P-PO_4^{-3} (mg L^{-1})	0.35 ± 0.26	0.44 ± 0.22

The shrimp performance parameters of *L vannamei* during experimental period of 105 days (Table 2).

temperature decreased during the culture period, due to local latitude (32 °S) and it reached the mean value of 19.4 °C in the last two weeks. In spite of the low WGR, the survival was above 80% in both treatments (Table 2). During the winter and in subtropical areas these animals reduce their food intake, as a result the growth declines (KUMLU *et al*., 2003; SOARES *et al*., 2012; BARBIERI *et al*., 2016).

The growth and survival of shrimps begin to decline when temperature is below 23 °C (WYBAN *et al*., 1995), and may cause mortality during the coldest months (PEIXOTO *et al*., 2003). Under the same experimental units of the current study, FRÓES *et al*. (2012) worked in intensive system and stocking density of 85 shrimp m^{-2}. The shrimp performance (productivity 8.7 t ha^{-1} cycle, FCR 1.22, final weight 10.7 g, WGR 0.63 g $week^{-1}$) were similar to the ones in this experiment. The author informed the mean temperature (24.3 °C) did not provide the maximum growth potential of the shrimps. Although, FÓES *et al*. (2016) had previously used nursery and after transferred to the grow-out ponds (20 shrimp m^{-2}), they concluded that temperature (24 °C to 27 °C) caused the lower weekly growth (0.42 to 0.54 g). BOYD and CLAY (2002) used ponds lined with a bottom area of 650 m^2 to 1.6 ha, stocking densities between 80 and 160 shrimp m^{-2} and reached productivity of 14.1 ton ha^{-1} until 27.2 ton ha^{-1}. However, there was influence of temperature on the WGR values: 0.95 g $week^{-1}$ and 0.6 to 0.7 g $week^{-1}$ when the shrimps were stocked in the warmest months (27 °C) and coldest months (23 °C), respectively. For the others penaeids in traditional systems, some authors considered the lows temperatures responsible for reduction in shrimp performance. In the autumn, KRUMMENAUER *et al*. (2006) worked with *Farfantepenaeus paulensis* in cages at Patos Lagoon Estuary, located near the present study. Although the temperature (mean value of 17.5 °C) and the growth rate declined, they confirmed the possibility to apply between 40 and 120 shrimp m^{-2}. In the case of *Litopenaeus schmitti,* MÁRQUEZ *et al*. (2012) used 8, 20 and 50 shrimp m^{-2} in ponds and recorded low growth rate of 0.55, 0.33 and 0.36 g $week^{-1}$, respectively. They considered negative effect of temperature (mean value of 24.61). Also related to temperature, MENA-HERRERA *et al*. (2006) worked in earthen ponds (500 m^2) with different stocking densities (50, 60 and 70 shrimp m^{-2}) in the seasons of spring/summer and autumn/winter. The WGR values were 0.62-0.64 g $week^{-1}$ in the milder period (22-26 °C) and 0.85 to 1.33 g $week^{-1}$ in the warmest season (27-31 °C). Moreover, the authors observed a significant difference between the treatments only during the spring/summer season. They suggested that the temperature was more important than the stocking density when evaluating the shrimp growth.

The salinity was within acceptable ranges for the species, between 10 to 35 ppt (GUNALAN *et al*., 2010). Pumped water from the well had salinity of 17 ppt at the start of the experiment. As previously reported, the ionic profile is more important than salinity. Therefore, it is recommended to determine the adequate ionic composition (Na^+, Ca^{2+}, Mg^{2+}, K^+) because it contributes to the development of the organisms farmed (HOU *et al*., 2012).

Despite the proximity to the sea, the well water composition was lower for the ions Na^+ and Ca^{2+} in the first weeks. However, the ionic ratio was not compromised Na^+: K^+ (20: 1) and Ca^{2+}: K^+ (0.7: 1). The replacement of seawater in the ponds supplemented the lack of ions and increased the salinity. The same ionic ratio was described by ARANEDA *et al*. (2008), the Na^+: K^+ ratio (20.4: 1) was below optimum water and they indicate that the ratio can be more significant than individual ions concentrations. Other authors report that the regular Na^+: K^+ ratio (28: 1) can vary in 10 points for more or less and do not affect the osmoregulation (SOWERS *et al*., 2006) and LIU *et al*. (2014) suggested that Na^+: K^+ ratio among 23: 1 to 33: 1 seems the most appropriate proportion. ESPARZA-LEAL *et al*. (2016) tested salinity (2, 4, 8, 12, 16, 25 and 35) in association with BFT system. They observed that low ion concentrations (calcium, magnesium, and potassium) at low salinities reflected in the shrimp growth but no significant differences were observed among some treatments. HOU *et al*. (2012) showed that the growth performance of shrimp at high levels of Ca^{2+} concentration is required when there is a wide salinity fluctuation. Comparable to this study, VALENZUELA-MADRIGAL *et al*. (2017) worked in a greenhouse with *L. vannamei* and stocking density of 150 shrimp m^{-2} during 133 days. They used four different sources of well water, in which the source number one was similar to that of seawater (Na^+: K^+ 30.9: 1 and Mg^{2+}: K^+ 3.1: 1). This source showed the best shrimp performance (final weight 12.8 g; FCR 1.55 and survival 78.4%). The authors explained that potassium in all treatments was lower that seawater, indicating that most important ions are chloride and sodium. Finally, in this study, the ionic ratios Na^+: K^+ (20: 1), Ca^{2+}: K^+ (0.7: 1), Mg^{2+}: Ca^{2+} (0.19: 1)

Table 2. Mean values (±SD) of shrimp performance parameters of *L. vannamei* culture in the comparative experiment between two densities using the BFT system, well water and in a subtropical environment. Different superscripts in the same row indicate significant differences ($P < 0.05$).

Shrimp performance	Treatment 100 shrimp m^{-2}	Treatment 150 shrimp m^{-2}
Initial weight (g)	0.08 ± 0.03	0.08 ± 0.03
Final weight (g)	10.05± 1.41	10.50 ± 1.46
Weekly growth rate (WGR) (g $week^{-1}$)	0.67 ± 0.02	0.7 ± 0.11
Apparent feed conversion rate (FCR)	1.20 ± 0.04	1.32 ± 0.16
Survival (%)	97 ± 2.5	88 ± 18
Productivity (kg ha^{-1})	9,748.52 ± 173^a	13,860.22 ± 707^b

and Mg^{2+}: K^+ (0.13: 1) were lower than sea water Na^+: K^+ (28: 1), Ca^{2+}: K^+ (1.08: 1), Mg^{2+}: Ca^{2+} (3.4: 1), Mg^{2+}: K^+ (3.6: 1) but it did not affected the shrimp performance. This can be checked by the following values of 100 shrimp m^{-2} (final weight 10.05 g; FCR 1.20 and survival 97%) and 150 shrimp m^{-2} (final weight 10.5 g; FCR 1.32 and survival 88%).

CONCLUSION

The BFT system provides a culture environment that enables the use of high densities. In southern Brazil, the stocking densities 100 and 150 shrimp m^{-2} were suitable showing efficient values of growth, survival and feed conversion of shrimps. However, the density of 150 shrimp m^{-2} was more profitable due to its higher productivity. The use of well water is an alternative. In this study, ionic ratio was lower than the values of seawater, but it did not affect the shrimp performance. With respect to location, it should be taken into account that climatic conditions in this area allow shrimp culture in ponds during the warmest months.

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