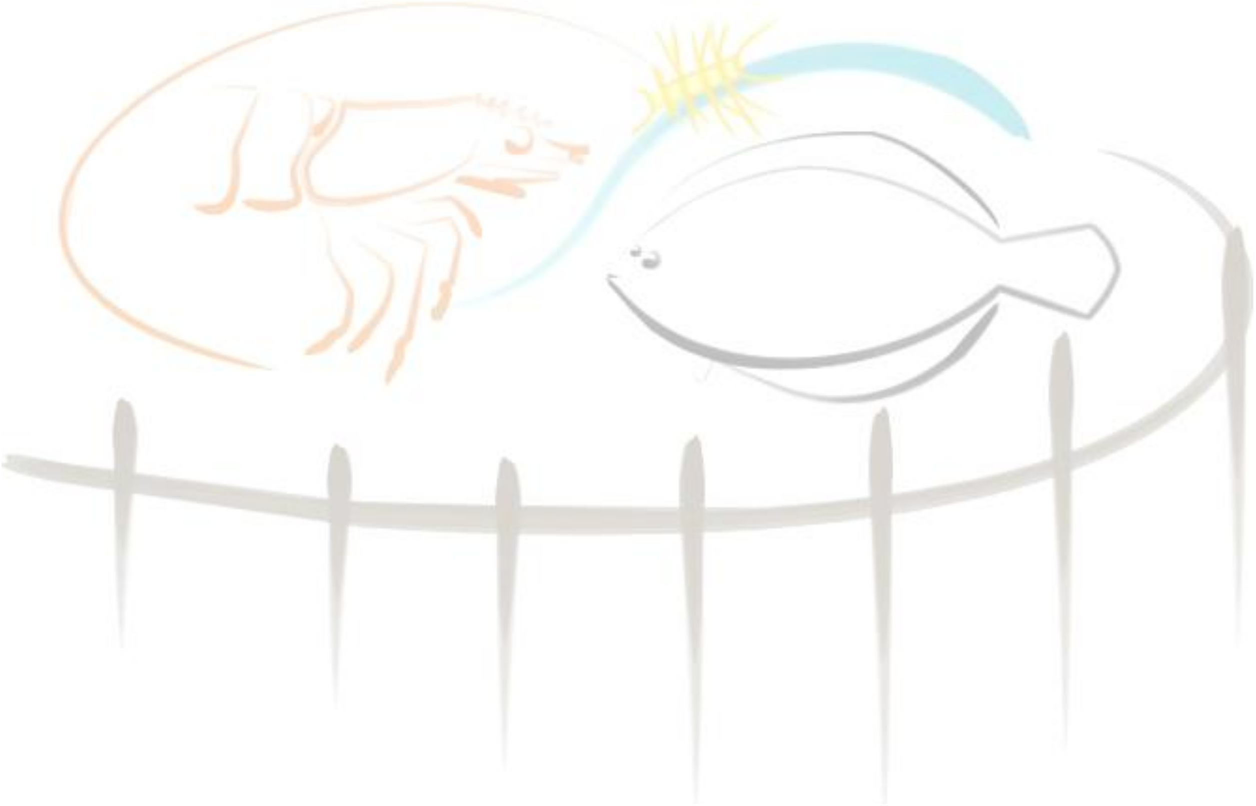
UNIVERSIDADE FEDERAL DO RIO GRANDE
INSTITUTO DE OCEANOGRAFIA
PROGRAMA DE PÓS-GRADUAÇÃO EM AQUICULTURA



MANEJO ALIMENTAR DE *Litopenaeus vannamei* CULTIVADO EM SISTEMA DE BIOFLOCOS: EFEITOS DA RESTRIÇÃO ALIMENTAR E DIFERENTES TAXAS DE ARRAÇOAMENTO SOBRE OS PARÂMETROS ZOOTÉCNICOS

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Rio Grande, 2016

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Manejo alimentar de *Litopenaeus vannamei* cultivado em sistema de bioflocos: efeitos da restrição alimentar e diferentes taxas de arraçoamento sobre os parâmetros zootécnicos

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Tese apresentada como parte dos requisitos para obtenção do grau de Doutora em Aquicultura no Programa de Pós-Graduação em Aquicultura da Fundação Universidade Federal do Rio Grande

Rio Grande, RS
Agosto de 2016

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DEDICATÓRIA

Dedico esta tese a meu Martin, que nem chegou ainda, mas me acompanhou durante esses últimos sete meses de experimentos, escritas e superação. Dedico também a todos os amigos que me ajudaram a concluir mais esta etapa.

AGRADECIMENTOS:

Primeiramente aos órgãos de fomento CNPq, CAPES, FAPERGS e FINEP, que desde a minha graduação, permitiram a realização da minha trajetória acadêmica. Certamente hoje, o Brasil se destaca em algumas áreas da pesquisa devido ao bom uso dos recursos financiados, mesmo que muitas vezes escassos.

Ao meu orientador Prof. Dr. Wilson Wasielesky Jr. (Mano) pela jornada de mais de 10 anos, por todos os ensinamentos, amizade, compreensão e apoio. Por sempre, além de um profissional exemplar, ser o suporte de muitos dentro da Pós-Graduação em Aquicultura (inclusive eu!).

Ao meu co-orientador Prof. Dr. Luís Henrique Poersch (Mineiro) igualmente por todas as oportunidades que me proporcionou durante todos esses anos e pela amizade e companheirismo do dia-a-dia.

Aos demais membros da banca, especialmente ao Prof. Dr. Eduardo Ballester, por fazer parte desse momento importante e por contribuir com minha vida acadêmica desde o início.

Igualmente, a todos aqueles que ao longo dos anos sempre estivera por perto, principalmente aos “bonitões e bonitonas” do Projeto Camarão: Bárbara, Aline, Dariano, Geraldo, Carlos G., Plínio, Alessandro, Júlio, João, Joaquin, Mariana, Sabrina, e os já extraditados Gabriel e André. A todos os demais que passaram por nosso laboratório durante todo esse tempo em que pude compartilhar de ensinamentos e experiências. Aos que de alguma forma contribuíram (e muito!) para a realização dos experimentos: Sandro Fabres, Hermes Terra, Diango e Anderson, Alessandro, Marcelo Honda e “Las camaroneras” Bárbara e Aline.

Aos colegas e professores do PPGAq, que contribuíram muito com minha formação profissional e pessoal, em especial aos Profs. Dr. Paulo Abreu e Dr. Marcelo Tesser pelas importantes opiniões durante as etapas iniciais do meu doutorado. Aos amigos que estiveram presentes dentro e fora da EMA: Ricardo, Okamoto, Janaína, Victor, Denis, Mário Juninho e Mário Nemo, André e Vita, Ana Luiza, Tatiana.

Às amigas que de uma forma mais que especial participaram de grandes momentos na minha vida, algumas mais recentes, outras de mais tempo, algumas de mais perto outras mais distantes, mas que estiveram sempre presentes: Bárbara e Jéssica (roomies mais que especiais, irmãs de coração), Tamyris, Aline, Juliana e Simone.

À minha mãe que sempre me incentivou e deu apoio para estudar, meu maior exemplo de esforço e batalha. Aos meus irmãos e irmã que, apesar de eu ter escolhido um

caminho bem diferente do convencional sempre me deram apoio para seguir em frente. Infelizmente é impossível citar os nomes de todos que se fizeram presentes na minha longa trajetória por aqui. Amigos da faculdade, do mestrado, do doutorado, do Larus e do Cassino. Mas como dizem, o importante não é o tempo que as pessoas permanecem na sua vida, mas sim as marcas que elas deixam. Assim, só posso agradecer pelo monte de marcas que a vida se encarregou de deixar durante esse tempo. Sou muito grata a todos, de todo meu coração.

RESUMO

O alimento artificial é um dos principais gastos nos cultivos intensivos de camarão ao redor do mundo. No cultivo em sistema de bioflocos, a comunidade microbiana pode ser uma fonte suplementar de alimento, levando a uma possível redução nas quantidades de ração a serem adicionadas ao sistema. No entanto, não se sabe ao certo o quanto de ração pode ser economizado e se a utilização de diferentes técnicas de manejo alimentar pode contribuir para uma redução ainda maior nas quantidades de ração a serem ofertadas aos animais cultivados. Dessa forma, foram delineados quatro experimentos utilizando diferentes taxas de arraçoamento e diferentes técnicas de manejo alimentar a fim de se observar se os bioflocos podem contribuir com a redução das taxas de arraçoamento e como a restrição alimentar pode influenciar nos parâmetros de crescimento e sobrevivência de *Litopenaeus vannamei* cultivado em sistema de bioflocos. As taxas de arraçoamento de todos os experimentos foram calculadas considerando um crescimento semanal esperado de 1 g/semana e uma mortalidade estimada de 0,5%/semana. Nos experimentos 1, 2 e 4, cada tratamento correspondeu a uma taxa de arraçoamento diferente e cada uma dessas taxas correspondeu a uma taxa de conversão alimentar fixa. No terceiro capítulo a taxa de arraçoamento foi calculada utilizando uma taxa de conversão alimentar fixa de 1,45. Os experimentos foram realizados em tanques de 150 L, com densidades de estocagem entre 300 e 400 camarões/m³. O primeiro experimento teve como objetivo avaliar a utilização de diferentes taxas de arraçoamento durante um ciclo de cultivo de 60 dias de *L. vannamei* em sistema de bioflocos. Os tratamentos testados foram: T0 (sem adição de ração), T0.4, T0.8, T1.2, T1.6 e T2.0. O peso final (g) foi menor em T0 ($P < 0.05$), seguido por T0.4, T0.8 e T1.2 ($P < 0.05$), não apresentando diferenças entre T1.6 e T2.0 ($P > 0.05$). O tratamento sem adição de ração apresentou a menor sobrevivência ($P < 0.05$) e todos

os outros tratamentos tiveram sobrevivências acima de 80%, sem diferenças estatísticas entre eles ($P > 0.05$). Os camarões podem sobreviver por longos períodos no sistema de bioflocos com quantidade reduzida de alimento artificial (T0.4). Como não houve diferenças entre o T1.6 e T2.0, pode-se concluir que não há necessidade de se aumentar as taxas de arraçoamento a esse nível no cultivo de bioflocos, economizando cerca de 25% do total de ração a ser adicionado. O segundo estudo teve por objetivo analisar os efeitos de diferentes taxas de arraçoamento e posterior re-alimentação no cultivo de camarões em sistema BFT. A primeira fase do estudo (restrição alimentar) durou 21 dias, e os seguintes tratamentos foram testados: T0 (sem adição de ração), T0.3, T0.6, T0.9, T1.2, T1.5, T1.8 e T2.1. Na segunda fase (realimentação), as taxas de arraçoamento foram baseadas em uma taxa de conversão fixa de 1,45. Ao final da restrição alimentar, os tratamentos T0, T0.3 e T0.6 apresentaram os menores pesos finais ($P < 0.05$). A sobrevivência foi baixa somente em T0 nas duas fases do experimento. Na segunda fase, os tratamentos T0, T0.3 e T0.6 apresentaram crescimento compensatório parcial. O estudo indica que esta técnica de manejo permite uma economia de até 24,79% da ração a ser adicionada no cultivo. O terceiro estudo avaliou diferentes períodos cíclicos de jejum e realimentação. Cinco tratamentos foram testados: Controle, em que os animais foram alimentados diariamente; F5S2, 5 dias de alimentação seguidos por 2 dias sem receber ração, F3S4, 3 dias de alimentação seguidos por 4 dias sem receber ração, e F5S2-R e F3S4-R em que os dias de alimentação eram os mesmos citados acima, porém com redução das quantidades de alimento de acordo apenas com os dias em que eram alimentados. Ao final do estudo, os camarões dos tratamentos F5S2 e F5S2-R não apresentaram diferenças significativas comparados ao controle para sobrevivência, biomassa final, taxa de crescimento específico, taxa de conversão alimentar e produtividade ($P > 0.05$) e indicam crescimento compensatório completo, sendo justificado pelo aumento do consumo alimentar. O quarto experimento analisou a utilização de biofilme na redução das taxas de arraçoamento. Os tratamentos testados foram: T0 e T0+B (sem adição de ração, com e sem biofilme, respectivamente), T0.6 e T0.6+B, T1.2 e T1.2+B, T1.8 e T1.8+B. O estudo teve duração de 42 dias. Ao final do experimento, os camarões que foram cultivados sem adição de ração apresentaram os menores pesos finais e sobrevivências, independente da utilização de biofilme ($P < 0.05$). O tratamento T1.2+B não apresentou diferenças significativas entre T1.2, T1.8 e T1.8+B para os parâmetros de crescimento e alimentação. Os resultados permitem concluir que a presença de biofilme no tratamento T1.2+B representou uma economia de 35% do total de ração a ser oferecido. De acordo com os resultados obtidos na presente tese, pode-se concluir que a comunidade microbiana do sistema de bioflocos pode representar uma importante fonte suplementar de alimento, reduzindo custos de operação e melhorando a sustentabilidade do sistema de cultivo.

ABSTRACT

The artificial food is one of the major costs in intensive shrimp farms around the world. In biofloc culture systems, the microbial community can be an additional source of food, leading to a possible reduction in the quantity of feed to be added to the system. However, it’s until uncertainly how much food can be saved and if the use of different management feeding techniques can contribute to a further reduction in the quantities of feed to be offered to reared animals. Thus, were designed four experiments using different feeding rates and different techniques of feed management in order to observe if the bioflocs can contribute to the reduction of feeding rates and how food restriction may influence the growth parameters and survival of *Litopenaeus vannamei* grown in biofloc culture system. Feeding rates of all experiments were calculated considering an expected weekly growth rate of 1 g / week and an estimated mortality of 0.5% / week. In experiments 1, 2 and 4, each treatment corresponded to a different feeding rate and each of these rates corresponded to a fixed food conversion ratio. In the third chapter the feeding rate was calculated using a fixed food conversion ratio of 1.45. The experiments were performed in tanks of 150 L, with stocking densities between 300 and 400 shrimp/ m^3. The first experiment evaluated the use of different feeding rates during a grow-out cycle of 60 days of *L. vannamei* in biofloc system. The treatments were: T0 (without addition of feed), T0.4, T0.8, T1.2, T1.6 and T2.0. The final weight (g) was lower at T0 ($P < 0.05$), followed by T0.4, T0.8 and T1.2 ($P < 0.05$), with no significant differences between T1.6 and T2.0 ($p > 0.05$). The treatment with no artificial feed addition presented the lowest survival ($P < 0.05$) and all other treatments had survivals of over 80%, with no statistical differences between them ($P > 0.05$). The shrimp can survive for long periods in biofloc system with reduced amount of artificial food (T0.4). Since there were no differences between T1.6 and T2.0, it can be concluded that there is no need to increase feeding rates at this level in biofloc shrimp culture, saving about 25% of the total feed to be added. The second study aimed to analyze the effects of different feeding rates and subsequent re-feeding on shrimp growth in BFT system. The first phase of the study (food restriction) lasted 21 days and the following treatments were tested: T0 (without addition of feed), T0.3, T0.6, T0.9, T1.2, T1.5, T1. 8 and T2.1. In the second phase (re-feeding), feeding rates were based on a fixed conversion rate of 1.45. At the end of the food restriction, treatments T0, T0.3 and T0.6 had the lowest final weights ($P <0.05$). Survival was lower only in T0 in the two phases of the experiment. In the second phase, the treatments T0, T0.3 and T0.6 had partial compensatory growth. The study indicates that this management technique allows savings of up to 24.79% of the feed to be added in culture. The third study evaluated different cyclical periods of fasting and re-feeding. Five treatments were tested: Control (fed intermittently); F5S2 (5 feeding days followed by 2 starvation days); F3S4 (3 feeding days followed by 4 starvation days). In these two treatments it was supplied the same amount of feed of the control group; F5S2-R and F3S4-R (the same feeding schedules but with reduced feed). At the end of the study, shrimp from treatments F5S2 and F5S2-R showed no significant differences compared to the control for survival, final biomass, specific growth rate, conversion rate, food and productivity ($P > 0.05$) and indicate full and partial compensatory growth, justified by the increase in food consumption. The fourth experiment evaluated the use of biofilm in reducing feeding rates. The treatments were: T0 and T0 + B (without addition of feed, with and without biofilm, respectively), T0.6 and T0.6 + B, T1.2 and T1.2 + B, T1.8 and T1. 8 + B. The study lasted 42 days. At the end of the experiment, shrimp that have been grown without the addition of feed had the lowest final weights and survival, regardless of the use of biofilm ($P <0.05$). The T1.2 + B treatment showed no significant differences

between T1.2, T1.8 and T1.8 + B for the growth parameters. The results indicate that the presence of biofilm in T1.2 + B treatment represented a saving of 35% of total feed being offered. According to the results obtained in this thesis, it can be concluded that the microbial community provided by the bioflocs and biofilm may represent an important additional food source, reducing operating costs and improving the sustainability of the culture system.

INTRODUÇÃO GERAL:

Apesar de apresentar variações em relação à produção e ao mercado, a carcinocultura ainda ocupa um lugar de destaque entre os grupos aquáticos mais importantes produzidos no mundo. Nos últimos anos, perdas consideráveis têm sido reportadas devido ao surgimento de doenças, sendo esse um dos pontos mais importantes no desenvolvimento de tecnologias com maior biossegurança (FAO, 2016; De Schryver et al., 2014).

Nos cultivos semi-intensivos e intensivos, há um incremento das densidades de estocagem, o que leva a um aumento nas quantidades de alimento artificial a serem adicionadas às unidades de cultivo e, potencialmente, um aumento das taxas de conversão alimentar no sistema (Peterson e Walker, 2002). Essa intensificação também pode ter como consequência um aumento da quantidade de nutrientes (principalmente nitrogênio e fósforo) a serem liberadas como efluente, causando potenciais riscos ao meio ambiente (Barraza-Guardado et al. 2013).

Além disso, outra questão que ainda é bastante apontada como um dos principais impactos negativos causados pela aquicultura é a utilização de farinha e óleo de peixe para a fabricação de rações, o que depende de recursos marinhos naturais. Naylor et al. (2009), indicam que a redução dessa dependência pode ser alcançada através da melhora na eficiência alimentar e com a substituição de farinha e óleo de peixe por ingredientes alternativos. Ainda, segundo esses mesmos autores, esse incremento na eficiência alimentar leva à redução das taxas de conversão alimentar e pode ser alcançado por meio de um manejo alimentar mais controlado.

De acordo com (Subasinghe et al., 2009), para que a atividade aquícola siga em expansão nos próximos anos são necessários avanços tecnológicos para que a produção aumente sem comprometimento dos recursos naturais aquáticos e terrestres, tornando a atividade sustentável nos aspectos ambientais, sociais e econômicos.

Dentro desse contexto, Crab et al. (2012) indicam que a tecnologia de cultivo em sistema de bioflocos pode cumprir com os três pré-requisitos básicos que tornam a aquicultura sustentável: produzir mais sem aumentar o uso de recursos; desenvolver sistemas que causem menos danos ao ambiente e, melhorar a relação custo-benefício, visando a sustentabilidade social e econômica. Esse sistema é caracterizado pela elevação das densidades de estocagem, juntamente com pouca ou nenhuma renovação de água, intensa aeração e adição de fontes de carbono para elevação da relação C:N na água, resultando na formação de uma comunidade microbiana diferenciada (Avnimelech e Kochba, 2009; Krummenauer et al., 2014, 2011; Suita et al., 2015).

Essa comunidade microbiana é formada por diferentes grupos de micro-organismos que, ao longo do tempo de cultivo apresentam variações e sucessões, e contribuem com dois aspectos principais no ambiente de cultivo. O primeiro é a manutenção da qualidade de água, através da metabolização dos compostos nitrogenados que seriam tóxicos para os animais cultivados, reduzindo ou anulando a necessidade de renovação de água (Ray et al., 2011; Vinatea et al., 2010). O segundo ponto importante da manutenção da comunidade microbiana é que a mesma pode servir como alimento suplementar para os animais cultivados, levando a melhores taxas de conversão alimentar, redução da necessidade de proteína nas rações, e, consequentemente reduzindo os gastos com alimentação, que é um dos principais custos na produção (Braga et al., 2016; Correia et al., 2014; Wasielesky et al., 2006).

Muitas pesquisas reportam que nesses sistemas há um incremento no desempenho de crescimento de camarões, em diferentes fases do cultivo, devido à disponibilidade de alimento *in situ* 24 horas por dia (Arnold et al., 2009; Emerenciano et al., 2012; Wasielesky et al., 2006; Xu e Pan, 2012). Essa maior disponibilidade de alimento natural contribui com a nutrição dos animais, sendo que já foram realizados estudos comprovando melhorias na utilização da ração, digestão de proteínas e melhor retenção proteica na biomassa dos camarões, podendo reduzir custos com alimentação e dependência por farinha de peixe, bem como redução dos impactos ambientais (Xu e Pan, 2012). Nesse sistema, sabe-se que a sobra de ração pode ser incorporada na forma de biomassa dos camarões através da reciclagem de nutrientes que é feita pela comunidade microbiana, assim a proteína que entra no sistema é consumida pelos camarões duas vezes: a primeira forma é através da ração, e em um segundo momento através da proteína microbiana. (Azam et al., 2002; Avnimelech 2012; da Silva et al., 2013).

De acordo com Burford et al. (2004), o consumo dos bioflocos pode contribuir com 20-30% da nutrição proteica de *Litopenaeus vannamei.* Em outro estudo, Cardona et al., (2015), observaram que juvenis do camarão *L. stylirostris* podem utilizar de 37 a 40% da produtividade natural do sistema de bioflocos como alimento. Além disso, sabe-se que o perfil da composição nutricional dos agregados microbianos, apesar de variável, pode representar uma importante fonte proteica e de outros nutrientes essenciais, já que são compostos por organismos vivos que fariam parte da alimentação dos camarões em ambientes naturais (Ballester et al., 2010; Maicá et al., 2014).

Assim, com a crescente preocupação com o consumo de ração e suas implicações para a sustentabilidade da aquicultura, bem como os gastos gerados pela alimentação, alguns protocolos de alimentação vêm sendo utilizados por produtores e pesquisadores. Esses protocolos, como por exemplo, o de Jory et al. (2001), apesar de serem desenvolvidos para cultivos intensivos de camarões e levarem em consideração diferentes fases de cultivo bem como variáveis como a temperatura da água, não levam em consideração a produtividade natural do sistema. Outra metodologia, proposta inicialmente por Garza de Yta et al. (2004), considera a produtividade natural em viveiros, com densidades de até 35 camarões/m². No entanto, atualmente, essa metodologia vem sendo utilizada também para sistemas de cultivo em bioflocos (Braga et al., 2016; Prangnell et al., 2016), desde que se conheça previamente o potencial de crescimento dos animais cultivados (taxas de crescimento semanais), bem como as taxas de conversão alimentar esperadas para seu sistema.

De acordo com Niu et al., (2016), a determinação de uma taxa de alimentação diária ideal é útil para minimizar o desperdício de alimento, reduzir a poluição da água e diminuir o custo de produção aquícola. O adequado manejo alimentar juntamente com taxas de alimentação ideais possuem dois objetivos principais. O primeiro é incentivar o rápido consumo, reduzindo assim a lixiviação e desperdício de nutrientes, e o outro é o de proporcionar maior potencial de crescimento através da minimização dos custos metabólicos. Tacon (1996), recomendou que experimentos de manejo alimentar devam ser realizados sob condições que estão perto de, ou simulam condições comerciais de produção para garantir a relevância dos resultados para a indústria de cultivo de camarão. No entanto, há poucos estudos quantificando a relação entre as taxas de arraçoamento e a taxa de crescimento dos camarões, principalmente em sistema de bioflocos, onde se deve considerar sempre a contribuição dos agregados microbianos na redução das quantidades de ração a serem fornecidas.

Aliando a importância de serem realizados estudos avaliando diferentes taxas de arraçoamento no sistema de bioflocos, outras técnicas de manejo alimentar podem ser utilizadas a fim de se incrementar o desempenho zootécnico dos animais, bem como os índices de eficiência alimentar. A utilização de técnicas de restrição alimentar por diferentes períodos com posterior realimentação dos animais cultivados pode contribuir para uma maior economia de ração no sistema (Wu e Dong, 2002; Zhu et al., 2016).

Normalmente, como consequência desses períodos de estresse alimentar, os animais cultivados podem apresentar algum grau de crescimento compensatório (Zhang et al., 2010; Zhu et al., 2016). A efetividade da compensação pode ser medida por meio da relação entre o tamanho e/ou peso dos animais que sofreram algum tipo de estresse e dos animais que seguiram submetidos às mesmas condições, ou condições controle. Assim, conforme Ali et al. (2003), diferentes graus de compensação podem existir, sendo eles: (1) compensação completa, quando os animais que sofreram alguma privação eventualmente atingem o mesmo tamanho e peso dos animais que seguiram continuamente sendo alimentados durante o mesmo espaço de tempo; (2) compensação parcial, quando os animais que passaram por restrição não atingem o mesmo tamanho e peso dos seus contemporâneos que não passaram por nenhum estresse, mas apresentam elevada taxa de crescimento, e podem apresentar melhores taxas de conversão alimentar durante o período de realimentação e; (3) sobre compensação, quando os animais que experimentaram restrição alimentar atingem peso e/ou tamanho maiores do que os animais controle. Ainda, segundo esses mesmos autores, o crescimento compensatório pode ser atingido devido a duas causas principais, hiperfagia e melhora na eficiência de conversão alimentar.

Assim, os animais têm seu crescimento acelerado devido ao aumento do consumo de alimento artificial ou a um melhor aproveitamento do alimento fornecido durante o período de realimentação. Esses dois fatores são as principais causas do melhor desempenho zootécnico que os animais podem apresentar após esses períodos. No sistema de bioflocos, alguns estudos reportam a existência de crescimento compensatório sob diferentes condições. Wasielesky et al. (2013) reportam que a realização de berçário de *L. vannamei* em diferentes densidades de estocagem e sua posterior re-estocagem na engorda em densidades mais baixas pode alavancar o crescimento compensatório. Esses autores indicam que em 20 dias os juvenis conseguem atingir compensação completa e que o sistema de bioflocos pode contribuir com essas elevadas taxas de crescimento, dando melhores condições para os camarões mesmo sob condições de estresse provocadas pelas elevadas densidades de estocagem. Em outro estudo, Hostins et al. (2015), submetendo pós-larvas de *Farfantepenaeus brasiliensis* a diferentes temperaturas durante a fase de berçário também verificaram a compensação do crescimento dos animais quando os mesmos saíam da condição estressora e eram cultivados dentro das temperaturas ótimas para a espécie.

No entanto, não há reportes da utilização de períodos de restrição alimentar e posterior realimentação no sistema de bioflocos, levando-se em consideração que o suprimento alimentar *in situ* provido pelos agregados pode ser uma importante fonte de alimento, e possivelmente, venha a contribuir ainda mais com os parâmetros de eficiência alimentar e consequentemente leve à redução das quantidades de alimento artificial a serem fornecidas durante o cultivo.

Além dos benefícios providos pela comunidade microbiana suspensa na água no cultivo de bioflocos, outra fonte de alimento natural nas unidades de cultivo pode estar aderida a substratos na forma de biofilme (perifíton). O biofilme comprovadamente contribui com a qualidade de água e com melhores resultados de desempenho zootécnico de diferentes espécies de crustáceos, devido a sua composição nutricional que é influenciada pelos microorganismos presentes nos substratos (Arnold et al., 2009; Schveitzer et al., 2013; Viau et al., 2012). Conforme Abreu et al. (2007), o biofilme por si só pode suprir mais de 70% do nitrogênio utilizado no crescimento de *F. paulensis*, possibilitando a redução da quantidade de alimento artificial a ser adicionado no cultivo dessa espécie. Da mesma forma, segundo Ballester et al. (2007), o cultivo de *F. paulensis* na presença de biofilme foi significativamente beneficiado em termos de peso final, sobrevivência e biomassa produzida.

Em sistemas com pouca ou nenhuma renovação de água, combinando a tecnologia de bioflocos com utilização de biofilme, Becerra-Dórame et al. (2012) indicam que a presença de organismos autotróficos e heterotróficos juntamente com alimento artificial melhorou o peso final, sobrevivência e reduziu as taxas de conversão alimentar de *L. vannamei*. Em outro estudo, Ferreira et al. (2015) observaram maior peso final e taxas de crescimento específico em juvenis de *L. vannamei* cultivados na presença de biofilme em sistema de bioflocos. De fato, os microorganismos presentes no biofilme podem apresentar diferenças no perfil nutricional quando comparados ao sistema de bioflocos, já que a abundância e diversidade de determinados micro-organismos pode apresentar diferenças, levando a melhores resultados de crescimento quando utilizados em conjunto. Assim, espera-se que a utilização de ambas as tecnologias também possa contribuir para a melhor eficiência alimentar nesse sistema de cultivo.

OBJETIVOS:

Objetivo Geral:

A presente tese tem como objetivo aplicar a utilização de diferentes técnicas de manejo alimentar, a fim de se avaliar a contribuição dos agregados microbianos na redução das taxas de arraçoamento e na melhora do desempenho zootécnico do camarão *Litopenaeus vannamei* cultivado em sistema de bioflocos durante a fase de engorda.

Objetivos Específicos:

- Avaliar os efeitos de diferentes taxas de arraçoamento e da restrição alimentar por um período de 60 dias no desempenho zootécnico do camarão *L. vannamei* cultivado em sistema de bioflocos;
- Analisar os efeitos da realimentação dos camarões após um período de 21 dias de restrição alimentar e diferentes taxas de arraçoamento no sistema BFT;
- Avaliar a utilização de diferentes taxas de arraçoamento e utilização de biofilme em conjunto com o sistema de bioflocos no desempenho zootécnico de *L. vannamei;*
- Observar a ocorrência de crescimento compensatório dos camarões quando submetidos a períodos cíclicos de alimentação e restrição alimentar cultivados na presença de bioflocos;
- Estimar a contribuição dos bioflocos e do biofilme para a melhora na eficiência alimentar e no desempenho zootécnico dos camarões;
- Avaliar os efeitos da redução das taxas de arraçoamento sobre a composição da comunidade microbiana dos bioflocos.

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CAPÍTULO 1

CULTURE OF LITOPENAEUS VANNAMEI AT DIFFERENT FEEDING RATES IN A BIOFLOC TECHNOLOGY SYSTEM

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****Artigo submetido à revista Journal of the World Aquaculture Society***

Observação: Tabelas e figuras foram incluídas no corpo do texto para facilitar a leitura da tese, além de um resumo em português.

Cultivo de *Litopenaeus vannamei* em sistema de bioflocos com diferentes taxas de arraçoamento

Resumo

Neste estudo foi avaliado o desempenho zootécnico de *Litopenaeus vannamei* cultivado com diferentes taxas de arraçoamento em sistema de bioflocos. Um experimento de 60 dias foi conduzido em um sistema de recirculação com bioflocos, projetado para manter a mesma qualidade de água para os diferentes tratamentos. Dessa forma, camarões (0,83 ± 0,24 g) foram estocados em tanques de 150 L a uma densidade de 400 camarões/m³. As taxas de arraçoamento foram calculadas considerando um crescimento semanal esperado de 1 g/semana e uma mortalidade estimada de 0,5%/semana. Cada tratamento correspondeu a uma taxa de arraçoamento diferente e cada uma dessas taxas correspondeu a uma taxa de conversão alimentar fixa. Assim, os tratamentos testados foram: T0 (sem adição de ração), T0.4, T0.8, T1.2, T1.6 e T2.0. O peso final (g) foi menor em T0, seguido por T0.4, T0.8 e T1.2, não apresentando diferenças entre T1.6 e T2.0. O tratamento sem adição de ração apresentou a menor sobrevivência ($P < 0.05$) e todos os outros tratamentos tiveram sobrevivências acima de 80% ($P > 0.05$). Os camarões podem sobreviver por longos períodos no sistema de bioflocos com quantidade reduzida de alimento artificial (T0.4). Como não houve diferenças entre o T1.6 e T2.0 ($P > 0.05$), pode-se concluir que não há necessidade de se aumentar as taxas de arraçoamento a esse nível no cultivo de bioflocos.

Abstract

This study evaluated the growth performance of Litopenaeus vannamei reared at different feeding rates in a biofloc culture system. A 60-d study was conducted in a water biofloc recirculation system, designed to maintain the same water quality to the treatments. Shrimp (0.83 ±0.24 g) were stocked in 150 L-tanks at a density of 400 shrimp m^{-3}.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 FCR. Therefore, the treatments tested were: T0 (no artificial feed addition), T0.4, T0.8, T1.2, T1.6 and, T2.0. The final weight (g) was lower in shrimp in T0, followed by T0.4, T0.8 and T1.2 treatments, and did not differ between the T1.6 and T2.0 treatments. The T0 treatment had the lowest survival, all other treatments presented survival higher than 80%. The shrimp could survive for long periods in the BFT culture with reduced feed (T0.4). As there were no significant differences between the T1.6 and T2.0 treatments, it is possible to conclude that there is no need to increase the feed input to higher levels in biofloc systems.

Introduction

In semi-intensive and intensive shrimp production, one of the largest operating costs is related to shrimp feed. This cost ranges between 40 to 60% of the total economic budget in aquaculture facilities (Molina 2009; Quintero and Roy 2010). In recent decades, the intensification of the shrimp production has resulted in high stocking densities and, consequently, increases in feed inputs. These increases have led to increased feed conversion rates (Peterson 1999; Peterson and Walker 2002) and production costs. Therefore, feed management should be one of the main factors to carefully control to optimize production (Davis et al. 2006). One approach to reducing feeding costs and increasing the sustainability of the culture includes the manipulation and use of natural productivity as a supplementary food source (Azim and Little 2006).

The benefits of natural productivity in an aquaculture system vary according to the degree of intensification (extensive to intensive cultures), species cultured, and management practices (pond fertilization, feed management, feed composition). It is important for shrimp producers to take advantage of the microbial community throughout the shrimp growth cycle (Allan et al. 1995; Moriarty 1997; Roy et al. 2012; Bojórquez-Mascareño and Soto-Jiménez 2013; Martinez-Córdova et al. 2015).

The biofloc culture system is an emerging technology that promotes natural productivity in water with essentially two main objectives: (1) the improvement of water quality, where microorganisms (primarily heterotrophic and autotrophic bacteria) play an important role in recycling nitrogenous compounds (ammonia and nitrite), increasing the microbial biomass and retaining these compounds within the ponds or raceways; and (2) the contribution of microorganisms to shrimp nutrition; the microbial community (aggregates of bacteria, protozoans, microalgae, nematodes, rotifers, and others) may serve as supplementary food, being an in situ food source 24 hours per day, and/or stimulate digestive enzymes and food consumption by the cultured animals (Crab et al. 2012; Emerenciano et al. 2012; Xu and Pan 2012; Cardona et al. 2015). Natural productivity is promoted in the environment culture through the addition of organic fertilizers to maintain a C:N ratio between 15-20:1, stimulating the formation of a complex microbial chain that could present variations in their nutritional composition, particle size, and microorganism classes, among other factors, which can influence the consumption and retention of the biofloc in the shrimp biomass (Crab et al. 2010; Ekasari et al. 2014; Suita et al. 2015).

The effects of natural productivity on biofloc systems have been studied in recent years primarily in order to determine the contribution of the suspended particles to the nutrition of shrimp, the variation of protein content in aqua-feeds and the substitution of fishmeal by plant-based products. In previous study, Burford et al. (2004) found that up to 29% of the flocculated particles could be a component of the Litopenaeus vannamei daily food intake. In a similar study, Cardona et al. (2015) observed that Litopenaeus stylirostris juveniles obtained 37-40% of their food from the natural productivity of the biofloc culture systems. Wasielesky et al. (2006) evaluated the performance of L. vannamei in biofloc systems with varied protein levels in artificial feed and concluded that the suspended particulate matter could improve feed conversion rate (FCR) reducing the feed costs and inorganic waste in production. Moreno-Arias et al. (2016) concluded that it is possible to replace fishmeal with a vegetable meal mix in diets for a L. vannamei nursery in a BFT system without a loss in production. All of these studies have great importance in assessing the natural productivity provided by biofloc as a potential source for environmentally friendly technology. Although studies have corroborated the contribution of biofloc to shrimp nutrition, it is difficult to determine the quantity of artificial feed that can be reduced in the presence of natural productivity. Thus, studies with different feeding rates are necessary to observe if the microbial community in the biofloc can effectively reduce the quantity of artificial feed added to the shrimp culture. Therefore, the objective of the present study was to evaluate the effects of different feeding rates and no food addition conditions on shrimp performance in a L. vannamei biofloc culture system.

Materials and Methods

Location

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

Experimental design and feeding rates

To isolate the potential negative effects of different feeding rates on water quality, a water biofloc recirculation system was designed (Fig. 1). This system was designed to observe only the effects of different feeding rates and the contribution of the biofloc on the shrimp performance. The system contained eighteen 150-L tanks (microcosm tanks), each with a bottom area of 0.5 m^2 and individual aeration. All the tanks had water output driven by gravity into a return pipe, which led to the matrix 10 m^3-tank. 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 stocking of shrimp). The water was pumped back into the microcosm tanks with the aid of a pump that had a flow rate of 1.25 L min^{-1} per tank (12 exchanges per day). The water quality in the matrix and microcosm tanks was monitored prior to initiating the study in order to observe if the concentrations of these parameters were similarly maintained throughout the units.

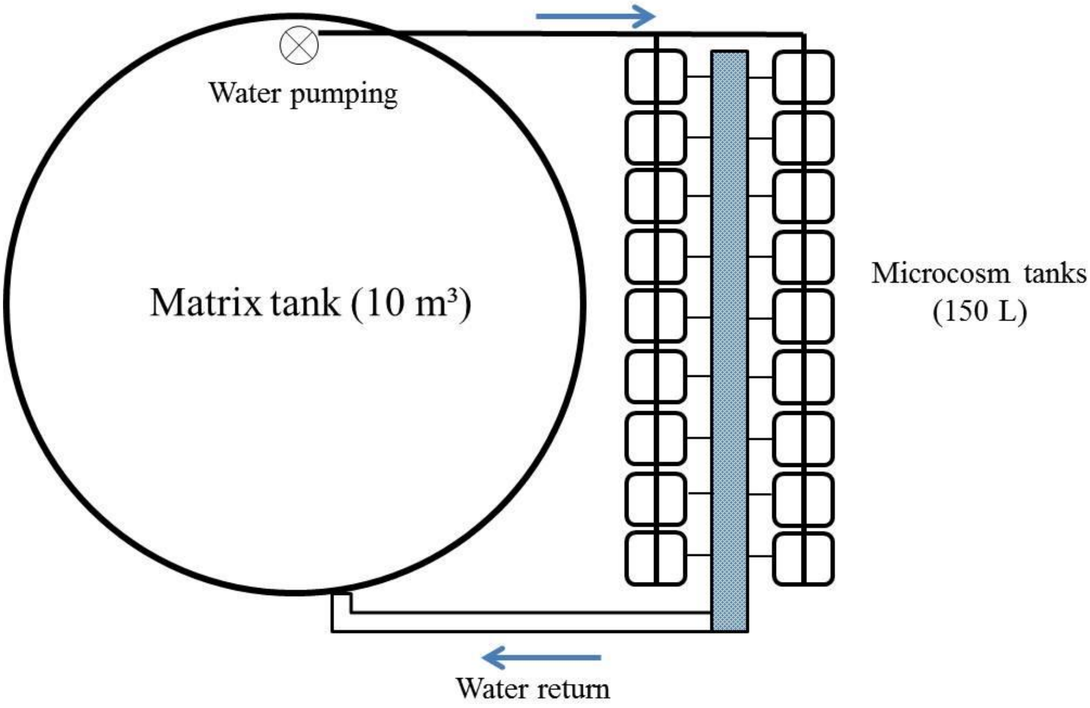

Figure 1 – Diagram representing the water biofloc recirculation system.

L. vannamei juveniles were stocked with an initial weight of 0.83 g (±0.24) at a stocking density of 400 shrimp m^{-3} (n=60). The duration of the experiment was 60 days.

The daily feed was calculated based on the methodology proposed by Garza de Yta et al. (2004) as follows:

Feed (daily) = (Number of Shrimp x Expected Weekly Growth Rate x Expected FCR)/7.

Shrimp were fed a commercial shrimp diet (Poti Active 38, 1.6 mm, D'Aguabi, Guabi Nutrição e Saúde Animal S.A., Campinas, São Paulo, Brazil). 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).

The feeding rates were calculated assuming different fixed FCRs and 1 g of growth per week, which is the mean value obtained in several cycles of L. vannamei cultured under similar conditions of the experiment at the Marine Station of Aquaculture. This expected weekly growth rate considered the shrimp genetics, stocking density, temperature and current feed quality available on the Brazilian market. Weekly, an estimated rate of 0.5% was reduced from the total number of stocked shrimp (estimated rate of natural mortality in the system) in each treatment to adjust the amount of feed supplied. Each different assumed FCR corresponded to a different feeding rate, i.e., an assumed FCR of 0.4 corresponded to the treatment T0.4, and so on. Thus, the treatments were T0 (no food addition), T0.4, T0.8, T1.2, T1.6 and T2.0. Weekly, biometrics (n=20) were performed in order to check the shrimp growth (weekly growth rates). At the end of the study all shrimp which remains in each tank were counted and weighted to evaluate the survival, productivity and food conversion ratio).

Culture monitoring

Daily, temperature (°C), dissolved oxygen (mg L^{-1}) and pH were monitored in the microcosm and matrix tanks using a digital multi-parameter meter (YSI Proplus, Yellow Springs, Ohio, USA). Weekly, total ammonia nitrogen (mg L^{-1}) (UNESCO 1983), nitrite (mg L^{-1}), nitrate (mg L^{-1}), phosphate (mg L^{-1}) (Strickland and Parsons, 1972), alkalinity (mg L^{-1}) (APHA, 1989), total suspended solids (mg L^{-1}) (Strickland and Parsons, 1972) and settleable solids (mL L^{-1}) (adapted from Avnimelech 2007) were measured in the matrix tank.

Organic fertilizations using molasses were performed only in the matrix tank to maintain a C: N ratio of approximately 15-20:1; these fertilizations followed the methodologies described by Avnimelech (1999) and Ebeling et al. (2006). Commercial probiotic (Sanolife Pro-W, Inve Aquaculture) was applied according the manufacturer's specification every 3 days. Similarly, the alkalinity was corrected according to Furtado et al. (2011) using hydrated lime, maintaining the concentration above 150 mg L^{-1} throughout the study.

Statistical analysis

The results were analyzed using a one-way ANOVA ($p < 0.05$). All tests were performed after confirmation of homogeneity of variances (Levene's test) and data normality (Kolmogorov-Smirnov test). Tukey's test was applied to detect significant among treatments (Sokal and Rohlf, 1969).

Results

The results of temperature (°C), dissolved oxygen (mg L^{-1}) and pH in the microcosm tanks are presented in Table 1. The mean temperature in these tanks ranged from 28.34 to 28.44°C and did not differ significantly among treatments ($p > 0.05$). The dissolved oxygen and the pH ranged from 6.03 to 6.14 mg L^{-1} and 7.75 to 7.78, respectively, and were not influenced by the different amounts of feed added ($p > 0.05$), which indicated that the efficiency of the biofloc recirculation system maintained the same water quality for the different feed treatments. The parameters monitored in the matrix biofloc recirculation tanks are presented in Table 2.

Table 1 – Water quality parameters monitored daily in the microcosm tanks during the 60-day study period.

Treatment	T0	T0.4	T0.8	T1.2	T1.6	T2.0
T (°C)	28.44 (±0.61)	28.37 (±0.64)	28.38 (±0.70)	28.34 (±0.78)	28.35 (±0.69)	28.39 (±0.64)
D.O. (mg L^{-1})	6.14 (±0.37)	6.05 (±0.35)	6.05 (±0.35)	6.04 (±0.38)	6.03 (±0.35)	6.03 (±0.35)
pH	7.78 (±0.14)	7.77 (±0.14)	7.76 (±0.14)	7.75 (±0.14)	7.75 (±0.14)	7.75 (±0.15)

Table 2 – Water quality parameters (mean ± standard deviation) of the matrix biofloc recirculation tank, monitored weekly during the 60-day study period.

Parameter	Mean ± SD
TAN (mg L^{-1})	0.04 (±0.05)
NO^{-}_2-N (mg L^{-1})	0.18 (±0.09)
NO^{-}_3-N (mg L^{-1})	99.06 (±25.69)
PO_4^{-3}-P (mg L^{-1})	0.76 (±0.23)
Alkalinity (mg $CaCO_3$ L^{-1})	153.61 (±17.64)
Salinity	28.53 (±1.98)
Total Suspended Solids (mg L^{-1})	482.86 (±135.73)
Settleable Solids (mL L^{-1})	22.78 (±43.52)

The growth parameters of the shrimp are presented in Table 3. The final weight (g) was lower in shrimp in the T0 treatment, followed by the T0.4, T0.8 and T1.2 treatments ($p < 0.05$), and did not differ significantly between shrimp from the T1.6 and T2.0 treatments ($p > 0.05$). Similarly, shrimp in the T0 treatment had the lowest survival rate among treatments (18.33±7.26%) ($p < 0.05$); all other treatments presented survival rates higher than 80%, and there was no significant difference among them ($p > 0.05$). The final shrimp productivity (kg m^{-3}) was lowest in the T0 treatment, followed by T0.4 ($p < 0.05$). The T0.8 treatment did not differ significantly from T1.2 ($p > 0.05$), and the mean values observed for T1.6 and T2 were statistically equal ($p > 0.05$). The weekly growth rates (g $week^{-1}$) were lowest in shrimp in the T0 and T0.4 treatments ($p < 0.05$), and the T0.8 and T1.2 growth rates were statistically equal ($p > 0.05$). The shrimp in the

T1.6 and T2.0 treatments also had no significant differences observed for this parameter ($p > 0.05$). The FCRs obtained at the end of the study, were lower in treatments where lower quantities of food were added. The FCR in the T0.8 treatment did not significantly differ from those calculated for shrimp in the T0.4 and T1.2 treatments ($p > 0.05$). The FCR for shrimp in the T1.6 treatment was equal to the FCRs calculated for the shrimp in the T1.2 and T2 treatments ($p > 0.05$), which was higher than all other treatments. Figure 2 shows the growth of shrimp in the different treatments over the 60-day study period.

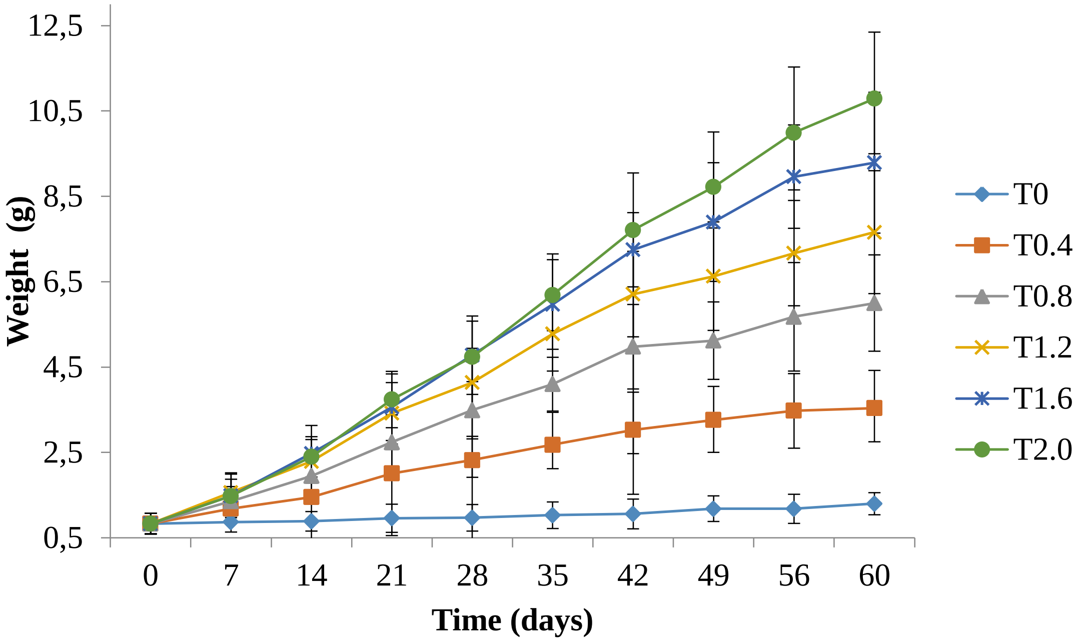

Figure 2 – Growth (g) of Litopenaeus vannamei in the biofloc system with different feeding rates over 60 days.

Table 3 – Growth parameters (mean ± standard deviation) of Litopenaeus vannamei reared in biofloc system with different feeding rates over 60 days.

Treatment	T0	T0.4	T0.8	T1.2	T1.6	T2.0
Initial Weight (g)	0.83 (±0.24)	0.83 (±0.24)	0.83 (±0.24)	0.83 (±0.24)	0.83 (±0.24)	0.83 (±0.24)
Final Weight (g)	1.30^{a} (±0.26)	3.54^{b} (±0.88)	6.00^{c} (±1.13)	7.66^{d} (±1.44)	9.29^{de} (±1.65)	10.79^{e} (±1.56)
Survival (%)	18.33^{a} (±7.26)	88.89^{b} (±9.18)	86.11^{b} (±9.18)	86.11^{b} (±4.19)	86.11^{b} (±0.96)	80.56^{b} (±8.39)
Productivity (kg m^{-3})	0.09^{a} (±0.03)	1.24^{b} (±0.17)	2.05^{c} (±0.11)	2.64^{cd} (±0.07)	3.20^{de} (±0.11)	3.48^{e} (±0.47)
WGR (g $week^{-1}$)	0.05^{a} (±0.09)	0.29^{b} (±0.22)	0.57^{c} (±0.34)	0.76^{c} (±0.34)	0.94^{d} (±0.41)	1.11^{d} (±0.48)
FCR Obtained	0^{a}	1.15^{b} (±0.15)	1.33^{bc} (±0.07)	1.55^{c} (±0.04)	1.71^{cd} (±0.06)	1.98^{d} (±0.28)

Values are the means of replicates ± standard deviation. Different superscripts in the same row indicate significant differences ($p < 0.05$).

Discussion

In L. vannamei culture systems, the water quality parameters should be maintained in an optimum range for the species in order to obtain better growth rates and efficiency in feed conversion rates and survival. According Ponce-Palafox et al. (1997), the best growth rates for L. vannamei are obtained at temperatures ranging from 25 to 35 ° C. In this study, the temperatures were maintained within this range in all treatments. For most species of shrimp, Van Wyk and Scarpa (1999) recommended dissolved oxygen concentrations above 5 mg L^{-1}; these values were maintained in all treatments. The pH values were maintained above 7.75 in all treatments, and the alkalinity levels remained above 100 mg L^{-1} throughout the duration of the experiment. According to Furtado et al. (2011) and Zhang et al. (2015), these values were within the range recommended for L. vannamei BFT culture systems, with no negative effects on growth rate, survival and biofloc maintenance. Similarly, salinity levels between 25 and 32 are likely more suitable for enhanced performance of L. vannamei in systems with low water exchange (Maicá et al. 2011; Maicá et al. 2014). The values observed in this study were within this range and did not negatively influence the growth parameters of the shrimp.

In biofloc culture systems, generally when an old inoculum of water is reused and this portion is dominated by nitrifying and/or heterotrophic bacteria, the nitrogenous compounds do not oscillate during the rearing period (Krummenauer et al. 2014). In addition, the form of nitrogen that tends to accumulate is nitrate ($N\text{-}NO^{-}_{3}$). In this study, total ammonia nitrogen (TAN) and nitrite ($N\text{-}NO^{-}_{2}$) did not reach levels that were toxic to the shrimp and did not negatively affect their growth and survival rates (Lin and Chen 2001; 2003). According to Furtado et al. (2011; 2015), concentrations of nitrate near 100 mg L^{-1} did not cause a reduction in food consumption or an increase in mortality of shrimp in super-intensive, low-water exchange culture systems. Therefore, the levels of nitrate observed in the study did not negatively influence the performance of the shrimp.

The concentrations of total suspended solids (TSS) and settleable solids (SS) are near the recommended levels suggested in most studies dealing with this subject and did not negatively influence the shrimp growth and feed consumption. The maintenance of TSS levels below 500 mg L^{-1} is recommended to avoid negative effects on other water quality parameters (reduction of dissolved oxygen, increase in nitrite concentrations) and does not adversely affect the shrimp physiology or feeding behavior (Samocha et al. 2007; Gaona et al. 2015).

It is well known that the natural productivity of a culture system is beneficial to cultured animals, independent of the intensity of the aquaculture (Porchas-Cornejo et al. 2011; Roy et al. 2012; Bojórquez-Mascareño and Soto-Jiménez 2013; Anrnold et al. 2015). In these studies, the authors confirmed that the consumption of natural food particles contributed to a reduction in artificial feed inputs, thereby reducing the FCRs and improving the profitability of the systems. The contribution of the natural productivity in water could vary according to the class size and species of the cultured animal, conditions of the culture system, composition of the artificial feed and assemblage of the microbial community (Focken et al. 1998; Martinez-Córdova et al. 1998; Burford et al. 2004; Soares et al. 2005; Wasielesky et al. 2006; Cardona et al. 2015). Although biofloc composition was known to be important to shrimp nutrition (Ballester et al. 2010), the previous studies on feeding rates were not completed under the same rearing conditions of the present study. In this trial, all shrimp were exposed to the same amount of natural productivity (biofloc) independent of treatment; however, the reduction in feed offered to animals caused a decrease in weight gain, thereby negatively influencing all performance parameters.

In the experimental conditions when no food was added, L. vannamei presented lower survival, final weight, productivity and minimal growth over the 60-day experimental period. These results differed from those observed by other authors who conducted similar studies without addition of feed but with much lower shrimp-stocking densities (Audelo-Naranjo et al. 2012; Roy et al. 2012). This finding indicates that the absence of artificial feed can lead to cannibalism at higher densities of shrimp, leading to poor survival rates. In the study of Wasielesky et al. (2006), better survival was observed in the absence of food (approximately 77%) in a similar stocking density of this study. However, this experiment lasted 20 days, during a period in which the animals were smaller and the natural biota corresponded to a large retention by the shrimp, probably leading to high survival rates (Burford et al. 2004). Emerenciano et al. (2012) investigated the application of biofloc technology as a food source in an intensive nursery of Farfantepenaeus brasiliensis and found no differences in final weight, final biomass, weight gain and survival between treatments with and without feed supply. In spite of the high stocking density utilized (1,000 shrimp m^{-3}), it is important to highlight that the trial lasted 30 days and studied PL_{25} shrimp, a life stage that is typically used in commercial productions. In this phase, small amounts of artificial feed were added, and the growth was based primarily on the natural productivity of ponds. Nevertheless, when a small amount of food was added to the system (T0.4), the survival rate was statistically equal to the treatments that received larger quantities of artificial feed even though lower growth rates were observed. This finding indicated that the shrimp could resist for long periods in the biofloc culture system with reduced feed and high densities when natural food was provided.

The lower FCRs were observed in treatments with decreased quantities of artificial feed. Nevertheless, these values were higher than the expected values at the beginning of the study, except for the predicted values of shrimp fed T2.0. In shrimp biofloc cultures, typically are expected lower FCRs that than observed in intensive cultures with no availability of natural food. However, the values obtained in this experiment were comparable to studies in similar culture conditions. Ray et al. (2010)

obtained FCRs of approximately 2 with a final stocking density of 328 shrimp m^{-3} in a complete culture cycle of L. vannamei during 12 weeks, meaning that productivities and weekly growth rates were very similar to those of the present study. The mean FCRs obtained by Krummenauer et al. (2011) in different stocking densities (150, 300 and 450 shrimp m^{-2}) were equally as close to those observed in this trial, with similar management techniques.

The calculation and adjustment of feed offered to shrimp were based on the formula primarily reported by Garza de Yta (2004) and have been used throughout the years in semi-intensive facilities with a good response in shrimp growth because it considers the natural productivity of the system. Samocha et al. (2007) adopted this calculation for biofloc culture systems, using a relative lower shrimp density (81 shrimp m^{-3}), and observed FCRs relatively similar to the values assumed at the start of the growth study. Therefore, this study affirms that this formula produces a good feeding strategy for various systems, including BFT systems. It is important to highlight that these calculations could be developed by each shrimp farm when the system operation is well-known and the major factors that influence the shrimp performance (genetics, water and feed quality, density) are considered.

In conclusion, according to the obtained results, there were no differences in final weight and survival between shrimp in the T1.6 and T2.0 treatments. These treatments resulted in similar values of productivity and weekly growth rates, indicating that there is no need to increase the feed input when the same quantity of natural food is provided with good water quality. Quantitatively, the difference represents approximately 25% of feed offered over the culture period. This value could represent a significant savings in terms of the cost of production as well as a reduction in negative environmental impacts. Moreover, the high survival rates observed in all treatments that received artificial food, independent of the quantity, enable us to state that the biofloc culture system (with high natural productivity and good water quality) is an efficient technique that allows the maintenance of shrimp in high stocking densities and with a small amount of feed for prolonged periods (over 60 culture days). The completion of additional studies using shorter periods of food restriction and the possible occurrence of compensatory growth is strongly encouraged to verify biofloc as an important supplementary food item under these conditions and the production of shrimp in a more sustainable system.

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CAPÍTULO 2

THE EFFECTS OF DIFFERENT FEEDING RATES AND RE-FEEDING OF *Litopenaeus vannamei* IN A BIOFLOC CULTURE SYSTEM

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Artigo submetido à revista Aquacultural Engineering

Observação: Tabelas e figuras foram incluídas no corpo do texto para facilitar a leitura da tese, além de um resumo em português.

Efeitos de diferentes taxas de arraçoamento e realimentação no cultivo de *Litopenaeus vannamei* em sistema de bioflocos

Resumo

A produtividade natural no Sistema de bioflocos pode ser uma importante fonte de alimento suplementar para os camarões, representando economia em alimento artificial. O objetivo do presente estudo foi avaliar os efeitos da utilização de diferentes taxas de arraçoamento por um período de 21 dias, com posterior período de realimentação em um sistema experimental de microcosmo na presença de bioflocos. Juvenis de *Litopenaeus vannamei* (1,14 ± 0,38g) foram estocados a uma densidade de 400 camarões/m³ em tanques de 150 L em um sistema de recirculação de água com bioflocos em duas fases. As taxas de arraçoamento foram calculadas considerando uma taxa de crescimento semanal esperada de 1 g/semana e uma mortalidade estimada de 0,5%/semana. Cada tratamento correspondeu a uma taxa de arraçoamento diferente e cada uma dessas taxas correspondeu a uma taxa de conversão alimentar fixa. A primeira fase do estudo (restrição alimentar) durou 21 dias, e os seguintes tratamentos foram testados: T0 (sem adição de ração), T0.3, T0.6, T0.9, T1.2, T1.5, T1.8 e T2.1. Na segunda fase (realimentação), as taxas de arraçoamento foram calculadas baseadas na média dos melhores resultados obtidos na primeira fase do estudo, a uma taxa de conversão fixa de 1,45. O período de realimentação durou mais 29 dias. Não foram observadas diferenças significativas para os parâmetros de qualidade de água entre os tratamentos ($P > 0.05$). Ao final da restrição alimentar, os tratamentos T0, T0.3 e T0.6 apresentaram os menores pesos finais ($P < 0.05$), e os pesos finais dos outros tratamentos não diferiram significativamente entre eles ($P > 0.05$). A sobrevivência foi baixa somente em T0 nas duas fases do experimento. Os outros tratamentos apresentaram taxas de sobrevivência acima de 95% sem diferenças significativas entre

eles ($P > 0.05$). O consumo alimentar não aumentou durante a fase de realimentação, indicando que não ocorreu hiperfagia após o período de restrição alimentar. Na segunda fase, as taxas de crescimento específico foram mais elevadas nos tratamentos que receberam as menores quantidades de ração na primeira fase, e os tratamentos T0, T0.3 e T0.6 apresentaram crescimento compensatório parcial quando comparados com os tratamentos com elevadas taxas de alimentação. O presente estudo indica que camarões podem ser cultivados em sistema de bioflocos utilizando técnicas de manejo alimentar com baixas taxas de alimentação, obtendo um crescimento compensatório parcial e elevadas taxas de sobrevivência, economizando até 24,79% da ração a ser adicionada no cultivo.

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 significant lower final weights ($P < 0.05$), and the weights in the other treatments does not significantly differ ($P > 0.05$). The survival rate was lower only in T0 in the two phases of the study ($P < 0.05$). 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.

Keywords: biofloc, feeding, compensatory growth, natural productivity, shrimp culture

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 et al. 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 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 can 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 can 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 can 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/m³ (n=60). The study lasted 50 days.

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. This phase lasted 21 days; 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).

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 m² and individual aeration. All tanks had gravity-driven water output into a return pipe leading to a matrix tank with a 20-m³. 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 daily feed was calculated based on the methodology of Garza de Yta et al. (2004). The feeding rates were calculated, assuming different food conversion ratios (FCR) and 1 g of growth per week. Each assumed FCR value to a different feeding rate. The following formula was used to calculate daily feeding:

Feed (daily) = (Number of Shrimp x Expected Weekly Growth Rate x Expected FCR)/7.

Shrimp were fed a commercial shrimp diet (Poti Active 38, 1.6 mm, D’Aguabi, Guabi Nutrição e Saúde Animal S.A., Campinas, São Paulo, Brazil). 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).

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 (Biopar Equipamentos Eletro-Eletrônicos Ltda., Model S150SD2) at 60°C until constant weight. Subsequently, the final dry weight was recorded using a digital balance with 0.001 g of precision

(Shimadzu, Model UX420H). Feed leaching and percent moisture of the artificial feed were measured prior to initiating the study to calculate the food conversion ratio and feed intake.

2.3. Data calculation:

The weights (n=20) were assessed weekly with analytical balance with 0.001g of precision (Marte Científica e Instrumentação Industrial Ltda., Model: AD2000) to determine the weekly growth rates. At the end of Phase 1, all shrimp were counted and re-stocked to verify the survival in each tank after food restriction. At the end of the study, all animals from each tank were counted and individually weighed.

Additionally, the following parameters were calculated to evaluate shrimp growth and feeding parameters in the two phases of the study: Specific growth rate in wet weight (SGR_w: % day^{-1}) = 100 x (lnW2 – lnW1)/t

Feed intake (FI: % body weight day^{-1}) = 100 x D_f / [t x (W1 + W2)/2]

Feed conversion efficiency in wet weight (FCE_w: %) = 100 x (W2 - W1)/D_f

Food conversion ratio (FCR) = (Final biomass – Initial biomass)/D_f

Productivity ($kg.m^{-3}$) = Final biomass (kg) – Initial biomass (kg)/volume of tank (m^3)

Where W2 and W1 are the final and initial wet weights (g) of the shrimp within a measured interval; t is the measuring interval (days); and D_f is the dry diet intake (g) within a measured interval.

2.4.Culture monitoring:

The temperature (°C), dissolved oxygen and pH in the microcosm and macrocosm tanks were monitored daily using a digital multiparameter meter (YSI Proplus, Yellow Springs, Ohio, USA). The total ammonia (UNESCO, 1983), nitrite, nitrate, phosphate (Strickland & Parsons, 1972), alkalinity (APHA, 1989) and total suspended solids (Strickland & Parsons, 1972) from the matrix tank were monitored weekly.

Organic fertilizations with molasses were conducted in the matrix tank to maintain a C:N relation of approximately 15-20:1, these fertilizations were performed according to Avnimelech (1999) and Ebeling et al. (2006). Commercial probiotic (Sanolife Pro-W, Inve Aquaculture) was applied according the manufacturer's specification every 3 days. Similarly, the alkalinity was corrected according to Furtado et al. (2011) using hydrated lime to maintain concentrations higher than 150 $mg.L^{-1}$ during the study.

2.5. Statistical analysis:

The results were analyzed using one-way ANOVA ($p < 0.05$). All tests were performed after confirming homogeneity of variances (Levene's Test) and data normality (Kolmogorov-Smirnov test). Tukey's test was applied to detect significant differences between treatments (Sokal & Rohlf, 1969).

3. RESULTS

The temperatures (°C), dissolved oxygen concentrations ($mg.L^{-1}$) and pH registered in the microcosm tanks over the 50 experimental days did not present significant differences between treatments ($P > 0.05$). The mean temperature ranged from 28.48 to 28.59°C, the dissolved oxygen concentration ranged from 6.67 to 6.82 $mg.L^{-1}$ and, the pH values were between 7.99 and 8.03 (Table 1). In the macrocosm tank, the mean total ammonia nitrogen (TAN) concentration was 0.06 (±0.05) $mg.L^{-1}$, the nitrite-nitrogen ($N\text{-}NO^{-}_{2}$) concentration was 0.02 (±0.01) $mg.L^{-1}$, the nitrate-nitrogen ($N\text{-}NO^{-}_{3}$) concentration was 29.94 (±7.76) $mg.L^{-1}$, the orthophosphate ($P\text{-}PO_4^{-3}$) concentration was 1.47 (±1.06) $mg.L^{-1}$, alkalinity was 161.89 (±21.33) $mg.L^{-1}$, salinity was 30.85 (±1.62), and the concentration of total suspended solids (TSS) was 245.83 (±66.70) mg.L^{-1}.

Table 1 – Water quality parameters (means ± standard deviation) monitored daily in the microcosm tanks during the 50 days of the study.

Treatment	T0	T0.3	T0.6	T0.9	T1.2	T1.5	T1.8	T2.1
T (°C)	28.50 (±0.85)	28.59 (±0.83)	28.55 (±0.83)	28.51 (±0.84)	28.55 (±0.86)	28.59 (±0.83)	28.48 (±0.83)	28.53 (0.86)
D.O. (mg.L^{-1})	6.89 (±0.49)	6.75 (±0.52)	6.82 (±0.50)	6.78 (±0.53)	6.81 (±0.52)	6.67 (±0.52)	6.75 (±0.53)	6.74 (±0.52)
pH	8.03 (±0.09)	8.01 (±0.09)	8.01 (±0.08)	8.02 (±0.08)	8.02 (±0.08)	7.99 (±0.09)	8.01 (±0.09)	8.01 (±0.08)

In table 2 are showed the specific growth rates (% day^{-1}), weekly growth rates (WGR), feed intake (% body weight day^{-1}), feed conversion efficiency (%), and food conversion ratios (FCR) in the two phases of the study. The results of SGR (%) in Phase 1 were significant lower in T0 and T0.3 ($P < 0.05$). The treatment with fixed FCR 0.3 did not present significant differences among SGR values in T0.6 and T0.9 ($P > 0.05$). The SGR in treatments T0.6 and T0.9 did not present significant differences among the other treatments in this phase ($P > 0.05$). In the re-feeding phase, the SGR was higher in T0 ($P < 0.05$). Treatment 0.3 did not show significant differences between T0 ($P > 0.05$) and among T0.6, T0.9, T1.2, and T1.5 ($P > 0.05$). The weekly growth rates in the first phase of the study were lower in T0 and T0.3, which did not significantly differ between them ($P > 0.05$). The T0.3 was statistically equal to T0.6 ($P < 0.05$), but differed from the other treatments ($P < 0.05$). The T0.6 treatment was equal to T0.9, T1.2, T1.5 and T1.8 ($P > 0.05$). Treatment T2.1 did not significantly differ from T1.2, T1.5 and T1.8 ($P > 0.05$). Furthermore, the weekly growth rates in the second phase of the study did not show significant differences among any treatment.

The feed intake was significantly different for all treatments in the first phase of the study ($P < 0.05$). In the second phase, the feed intake was significantly lower only in T0 ($P < 0.05$), and did not present differences among other treatments ($P > 0.05$). In the food restriction phase, the values of FCE (%) were statistically equal between T0 and T2.1 ($P > 0.05$). Treatment 2.1 did not show significant differences for the FCE values among T1.2, T1.5 and T1.8 ($P > 0.05$). T0.9, T1.2 and T1.5 did not show significant differences ($P > 0.05$). Higher FCE values were observed in treatment 0.3, followed by T0.6, and these values were significantly different between them and among other treatments ($P < 0.05$). In the second phase of the study, the highest FCE was observed in T0 ($P < 0.05$). The FCEs registered in other treatments were statistically equal ($P > 0.05$).

In the T0 treatment, the FCR obtained at the end of Phase 1 was significantly lower compared with the other treatments ($P < 0.05$). This parameter did not present significant differences among T0.3, T0.6 and T0.9 and was similar among T0.6, T0.9, T1.2 and T1.5 ($P > 0.05$). For T0.9 through T1.8, the FCR values were statistically similar ($P > 0.05$). The FCR obtained for T2.1 presented values similar to those for T1.8 ($P > 0.05$). At the end of the re-feeding phase, the FCR obtained for T0 was lower than the other treatments ($P < 0.05$), but there were no significant differences among any of the other treatments tested ($P > 0.05$). Calculating the global average of the FCR values (over the time of study) in each treatment it was revealed that T0 presented the lowest FCR ($P <0.05$), and for T0.3 through T1.8, this parameter did not significantly differ among treatments (P > 0.05). The T2.1 average FCR was statistically equal to T1.8 (P > 0.05) but significantly differed from all other treatments (P < 0.05).

Table 2 – Feed intake (% body weight.day^{-1}), Specific growth rates (% day^{-1}), feed conversion efficiency (%), feed conversion rates (FCR) and weekly growth rates (WGR) in Phase 1 (food restriction) and Phase 2 (compensatory growth) registered during the 50 experimental days.

Treatment	0	0.3	0.6	0.9	1.2	1.5	1.8	2.1
FI (%) Phase 1	0^{a}	1.81 (±0.01)^{b}	3.63 (±0,01)^{c}	5.39^{d} (±0.11)	6.57^{e} (±0.27)	7.85^{f} (±0.31)	8.96^{g} (±0.11)	10.57^{h} (±0.38)
FI (%) Phase 2	1.66^{a} (±0.22)	2.95^{b} (±0.07)	2.95^{b} (±0.07)	2.96^{b} (±0.06)	2.93^{b} (±0.03)	2.91^{b} (±0.03)	2.92^{b} (±0.06)	2.95^{b} (±0.07)
SGR (% day^{-1}) Phase 1	-0.03^{a} (±1.31)	2.83^{ab} (±2.30)	4.07^{bc} (±2.04)	4.88^{bc} (±1.81)	5.55^{c} (±1.52)	6.07^{c} (±1.22)	6.06^{c} (±1.31)	6.53^{c} (±2.03)
SGR (% day^{-1}) Phase 2	6.86^{c} (±1.22)	4.90^{bc} (±1.26)	4.33^{ab} (±1.23)	3.87^{ab} (±1.45)	3.35^{ab} (±1.20)	3.09^{ab} (±0.76)	2.94^{a} (±1.87)	2.75^{a} (±1.25)
FCE (%) Phase 1	0^{a}	348.87^{e} (±4.78)	170.53^{d} (±9.58)	115.91^{c} (±3.72)	90.53^{bc} (±5.61)	72.78^{bc} (±2.14)	65.09^{b} (±1.21)	55.76^{ab} (±2.30)
FCE (%) Phase 2	167.55^{b} (±5.64)	95.96^{a} (±4.25)	98.75^{a} (±5.80)	100.08^{a} (±5.03)	100.82^{a} (±1.06)	104.42^{a} (±1.92)	101.11^{a} (±6.59)	98.07^{a} (±2.52)
FCR Phase 1	0^{a}	0.88^{b} (±0.11)	1.09^{bc} (±0.14)	1.19^{bcd} (±0.04)	1.25^{cd} (±0.16)	1.30^{cd} (±0.06)	1.45^{de} (±0.11)	1.62^{e} (±0.12)
FCR Phase 2	0.44^{a} (±0.24)	1.08^{b} (±0.04)	1.07^{b} (±0.01)	1.09^{b} (±0.08)	1.10^{b} (±0.05)	1.08^{b} (±0.06)	1.18^{b} (±0.17)	1.17^{b} (±0.05)
FCR Global	0.85^{a} (±0.33)	1.05^{b} (±0.05)	1.07^{b} (±0.03)	1.12^{b} (±0.06)	1.15^{b} (±0.06)	1.16^{b} (±0.05)	1.28^{bc} (±0.08)	1.36^{c} (±0.07)
WGR(g/week) Phase 1	0.04^{a} (±0.05)	0.31^{ab} (±0.15)	0.51^{bc} (±0.16)	0.67^{c} (±0.09)	0.83^{cd} (±0.19)	0.97^{cd} (±0.26)	0.97^{cd} (±0.25)	1.04^{d} (±0.25)
WGR(g/week) Phase 2	1.20 (±0,60)	1.23 (±0.45)	1.24 (±0.25)	1.19 (±0.28)	1.14 (±0.22)	1.20 (±0.21)	1.10 (±0.38)	1.07 (±0.12)
WGR(g/week) Global	0.70^{a} (±0.76)	0.84^{ab} (±0.59)	0.93^{b} (±0.44)	0.97^{b} (±0.34)	1.01^{b} (±0.25)	1.10^{b} (±0.24)	1.05^{b} (±0.31)	1.06^{b} (±0.17)

The values are shown as the means of replicates ± standard deviation. Different superscripts in the same row indicate significant differences (P<0.05).

In table 3 are presented the initial and final weights (g), survival (S%) and productivity (kg.m^{-3}) observed at the end of the two phases of the study. In Fig. 1 is showed the growth of shrimp over the experimental period. At the end of the Phase 1 (food restriction), the treatment with no addition of artificial food (T0) presented a significantly lower final weight and survival rate ($P < 0.05$). The treatments with fixed FCR values of 0.3, 0.6 and 0.9 did not present significant differences in the final weights ($P > 0.05$). Treatment T0.9 did not show significant differences for final weight among other treatments in the first phase of the study, representing an intermediate value ($P > 0.05$). The survival at the end of the food restriction period was significant lower only in T0 ($P < 0.05$), and all other treatments had survival rates above 95% without significant differences among the treatments. At the end of the second phase of the study (re-feeding), the final weights in T0 and T0.3 were significant lower ($P < 0.05$; equal between them). Treatment 0.6 was not different from T0.9 and T1.2 ($P > 0.05$), and T0.9 did not present significant differences among the other treatments ($P > 0.05$). The survival rates observed at the end of the study were similar to those observed during the first phase, where T0 presented the lowest survival ($P < 0.05$) and the other treatments did not show significant differences for this parameter, as the survival among all treatments was higher than 94% in the re-feeding phase. The mean productivities achieved at the end of the study were lower ($P < 0.05$) in T0 (0.34 ± 0.20 kg.m^{-3}) and did not present differences among T0.3, T0.6 and T0.9 ($P > 0.05$). With respect to the final weight and survival, the productivity in T0.9 did not present significant differences among the other treatments at the end of the study ($P > 0.05$).

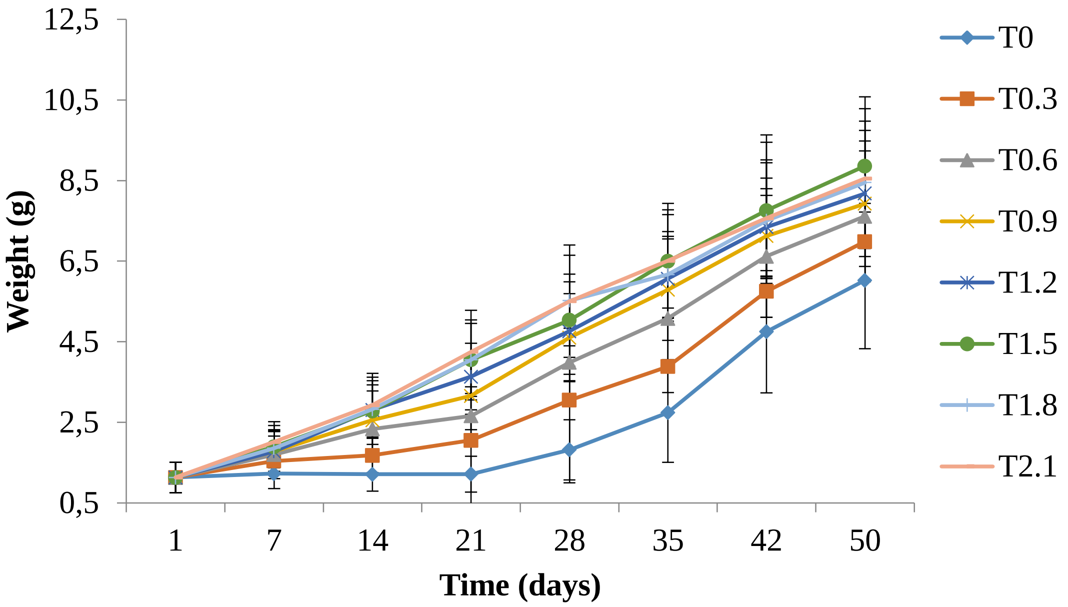

Figure 1 – Growth of shrimp (mean ± standard deviation) over the experimental period.

Table 3 – Initial weight (W_i), final weight (W_f) and survival (S%) in the two phases (food restriction and compensatory growth) and final productivity ($kg.m^{-3}$) after 50 days of study.

Treatment	$W_{initial}$ (g)	W_{final} (g) Phase 1	S (%) Phase 1	W_{final} (g) Phase 2	S (%) Phase 2	Productivity ($kg.m^{-3}$)
T0	1.14 (±0.38)	1.21^a (±0.45)	37.50^a (±1.18)	6.02^a (±1.69)	35.00^a	0.34^a (±0.20)
T0.3	1.14 (±0.38)	2.05^b (±0.65)	99.44^b (±0,96)	6.99^a (±1.44)	95.56^b (±2.55)	2.22^b (±0.12)
T0.6	1.14 (±0.38)	2.66^b (±0.72)	97.78^b (±3.85)	7.61^b (±1.62)	95^b (±3.33)	2.44^b (±0.17)
T0.9	1.14 (±0.38)	3.16^{bc} (±0.84)	98.89^b (±1.92)	7.93^{bc} (±1.56)	98.33^b (±4.41)	2.66^{bc} (±0.08)
T1.2	1.14 (±0.38)	3.63^c (±0.82)	98.33^b (±3.33)	8.18^{bc} (±1.56)	97.22^b (±3.47)	2.76^c (±0.17)
T1.5	1.14 (±0.38)	4.05^c (±0.99)	95.56^b (±0.96)	8.86^c (±1.72)	94.44^b (±5.36)	2.90^c (±0.20)
T1.8	1.14 (±0.38)	4.05^c (±0.90)	98.89^b (±1.92)	8.45^c (±1.52)	97.78^b (±2.55)	2.85^c (±0.16)
T2.1	1.14 (±0.38)	4.25^c (±1.03)	98.33^b (±3.33)	8.55^c (±1.74)	97.78^b (±2.55)	2.91^c (±0.19)

The values are presented as the means of replicates ± standard deviation. Different superscripts in the same column indicate significant differences ($P < 0.05$).

4. DISCUSSION

In shrimp culture, monitoring water quality parameters is fundamental to achieving good growth results and survival of the reared animals. The water quality can negatively influence food consumption, resulting in losses in production. The microcosm system designed in the present study was effective in maintaining the same water quality for shrimp in different treatments, and these parameters were maintained within the optimal levels for the growth and survival of the species. The experimental design showed efficiency in separating the effects of the different feeding rates utilized and the potential degradation of water quality. Furthermore, the system provided the same quantity of natural productivity (bioflocs) in all treatments, isolating the effects of the amount of suspended material in the nutrition of the shrimp.

The mean temperature, dissolved oxygen and pH recorded in the microcosm tanks were maintained within the levels recommended for *L. vannamei* (Ponce-Palafox et al. 1997; Van Wyk and Scarpa 1999; Furtado et al. 2011; Zhang et al. 2015). Similarly, the water quality parameters monitored in the macrocosm tank (alkalinity, salinity, total ammonia nitrogen, nitrite, nitrate, orthophosphate and total suspended solids) were maintained at optimum concentrations for shrimp culture, and did not negatively affect food consumption and the growth of the animals (Lin and Chen 2001; Lin and Chen 2003; Furtado et al. 2011; Maicá et al. 2014; Furtado et al. 2015; Gaona et al. 2016).

In studies of compensatory growth, SGR rates are frequently used to monitor the shrimp growth after a period of food restriction, and many of these studies associate elevated SGRs with full or partial compensation after a period of growth depression (Wasielesky et al. 2013; Hostins et al. 2015; Zhu et al. 2016; Fóes et al. 2016). At the end of the food restriction period, the lowest SGR and WGR values observed showed a decrease in quantity of artificial feed offered at some level. Upon re-feeding, however, the T0 and T0.3 treatments showed the highest values of SGR, and treatments with a higher quantity of artificial feed in the first phase of the experiment presented lower SGR values, showing that this parameter is primarily influenced by the feeding rate in shrimp biofloc culture. The WGR observed in the second experimental phase showed no significant differences among treatments, indicating a recovery pattern in treatments with feed reduced for a period of 21days. Although the T0.9 treatment presented a SGR numerically equal to T2.1, the observed WGR was lower in the first phase. However, the final weight did not show significant differences between these treatments, suggesting that the elevated FCE in this phase was responsible for achieving the same weight at the restriction period. A comparison of the SGR values and the final weights obtained at the end of the study revealed that even the SGRs calculated in T0 and T0.3 were higher in the second phase, but this was not sufficient to recover the weight that was not achieved in the first phase of the study. According to Ali et al. (2003), partial compensation occurs when the deprived animals fail to achieve the same size as the non-restricted contemporaries, but show rapid growth rates. In the present study, the treatments that were fed with lower rates (T0, T0.3 and T0.6) showed a partial compensation of the growth depression observed in the first period, indicating the occurrence of some degree of compensatory growth under these conditions.

In compensatory growth responses, hyperphagia and improvement in feed conversion efficiency are considered the main factors explaining the occurrence of the accelerated growth of the animals (Ali et al. 2003). In the first phase of the present study, the feed intakes were significantly different among treatments, and these rates were presumably higher in treatments receiving a higher quantity of artificial feed. However, at the end of the food restriction phase, the shrimp in groups receiving intermediate quantities of artificial feed (from T0.9 to T1.2) presented final weights similar to those observed with the higher feeding rate treatment (T2.1). Variations in the contribution of natural productivity for different size classes of shrimp explain this fact. According to Otoshi et al. (2011), *L. vannamei* grow better in the presence of natural productivity, and the contribution of pond water is more pronounced in the initial phases of grow-out. Roy et al. (2012) performed a series of studies in semi-intensive inland waters, reporting that small shrimp (initial weight of 0.25 g) receiving absolutely no commercial feed, obtained enough nutrition from pond primary productivity, and the growth of these animals was similar to that of the fed animals. However, in the re-feeding period, the feed intakes were lower only in T0, indicating that when experiencing long-term starvation, the shrimp reduce their overall consumption and potentially lose their capacities for recovery. The non-occurrence of hyperphagia in the second phase in other treatments (T0.9 and T1.2) is consistent with the theory that no compensatory growth responses occurred under these study conditions. Similarly, the increase in feed intake at the re-feeding period in the T0.3 treatment may reflect partial compensation in this treatment, as in T0, when the food offering initiated from the 22nd day until the end of the study accelerated growth.

Variations in food conversion efficiency have also been associated with compensatory growth responses under starvation and re-feeding regimes in different species of fishes and crustaceans (Wu and Dong 2001; Foss et al. 2009; Jiwyang 2010; Zhu et al. 2016). According to Quinton and Blake (1990), one effect of increasing the feeding rates is related to a decrease in the FCE values, indicating overfeeding. The results obtained in the present study are consistent with this idea, as lower FCE values were observed in the high feeding rate treatments during the first phase of the study. In the second phase, only the T0 treatment presented significantly higher FCE compared with the other treatments, indicating a potential effect of starvation on compensatory growth in this treatment.

Variations in the contribution of natural productivity in culture reflect a number of parameters that influence shrimp growth, such as the water and feed quality, the quantity and quality of natural productivity, the stocking density, shrimp genetics, management, the size of particles in water, the class size of the animals reared, and the system adopted (i.e., flow through or static) (Soares et al. 2005; Otoshi et al. 2011, Roy et al. 2012). Concerning the FCR, Wasielesky et al. (2006) observed a decrease from 1.39:1 to 1.03:1 when natural productivity was added to shrimp culture, indicating the potential to reduce the amount of feed in the presence of bioflocs. In the present study, the growth-enhancing factor that suggests that natural productivity is an important source of supplementary food is reflected in the reduction of the FCR values observed at the end of the culture period. Interestingly, in the second phase of the study, when the FCR value was fixed at 1.45, none of the treatments achieved this value, indicating improved feed utilization and growth rates higher than the estimated rates. In summary, at the end of the study, the calculated FCR values were much lower than the estimated values, even in treatments T1.8 and T2.1, which received a greater quantity of artificial feed, indicating the importance of natural productivity in shrimp growth.

Using the stable isotopes method, Burford et al. (2004) estimated that *L. vannamei* could obtain up to 29% of their daily food intake from flocculated particles. In a similar study, Cardona et al. (2015) observed that *Litopenaeus stylirostris* juveniles obtained 37-40% of their food from the natural productivity of the biofloc culture systems. Calculating the amount of feed consumed for the different treatments that did not differ in the main production parameters revealed that up to 24.79% of the feed could be saved. This value is similar to the findings of the studies mentioned above, suggesting that this percentage reflects the natural productivity promoted in the biofloc culture system. Thus, natural food items could represent significant cost savings, as feed is one of the most expensive items in shrimp farming.

Considering physiological, nutritional, immunological and behavioral factors, it is expected that shrimp under food restriction present lower weights and survival than animals that did not experience any feeding stress. In periods of starvation, the energy reserves are directed to support vital processes at the expense of the growth of the animals (Sánchez-Paz et al. 2006). According to Lin et al. (2012), a period of 14 days of starvation is sufficient for *L. vannamei* to lose their ability to return to the baseline values of immune parameters, and even with a re-feeding period of 5 days, the animals did not retrieve immunity, indicating immune fatigue. In addition, stocking densities above 100 shrimp m^{-2} could compromise the access to food in feeding trays (Costa et al. 2016). The poor growth in the T0, T0.3 and T0.6 treatments in the first phase indicated that the privation or reduction in the access of artificial food resulted in decreased final weight, reflecting the sum of the negative effects of the factors described above. At the end of the re-feeding phase, animals from these three treatments did not reach the same weight observed in other treatments, indicating that food restriction for 21 days negatively impacted the growth of shrimp, leading to poor productivity.

Typically, the point-of-no-return (PNR) and the point-of-reserve-saturation (PRS) are methods used to evaluate the resistance and recovery of animals to survive during food deprivation and re-feeding periods. Zhang et al. (2009) examined *Fenneropenaeus chinensis* juveniles and reported that a period of 7.86 days was sufficient to obtain 50% mortality under starvation. In the present study, the total absence of artificial feed in the first phase negatively affected the survival of shrimp, indicating that shrimp did not survive under the natural productivity provided by bioflocs. Audelo-Naranjo et al. (2012) grew *L. vannamei* for 42 days at different stocking densities with no food addition and no water exchange, and obtained better results than those observed in the present study. Moreover, Roy et al. (2012) reared shrimp with no artificial food addition, observing survival rates higher than the values observed in the present study (poor survival was 61%). In the present study, the stocking density corresponded to approximately 10% of the densities tested by the authors cited above, and this factor likely reflects the lower survival observed in the T0 treatment. The high survival rates observed in treatments with low rates of feeding (T0.3 and T0.6) indicate the potential to grow shrimp with reduced feeding amounts for a period of 21 days without compromising their survival. This observation is important in terms of farming management, when a producer may use this strategy. The shrimp also showed partial growth compensation after this period of food restriction.

In conclusion, although there was observed partial compensatory growth after a period of food restriction, the results obtained in the present study are important in terms of feeding management, suggesting the potential to grow shrimp in a biofloc system with high stocking densities and reduced feeding inputs. The periods of food restriction and re-feeding did not affect the growth of the shrimp. Combining good water quality, high quality of artificial feed, animals with good immunological conditions, and feeding strategies that consider the importance of natural productivity of the system, it is possible to estimate a reduced FCR and an increased weekly growth rate to calculate the amount of feed required to rear shrimp. In the light of the results of the present study, we recommend a minimum FCR of 1.1 and a maximum estimated weekly growth rate of 1.1 g week^{-1} to culture *L. vannamei* at a stock density of 400 shrimp m^{-3} in the presence of bioflocs. Notably, these values could vary according to the parameters described above, and farmers should develop proper feeding strategies considering the particular conditions of each farm.

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CAPÍTULO 3

CYCLICAL STARVATION AND RE-FEEDING PERIODS IN A *Litopenaeus vannamei* BIOFLOC CULTURE SYSTEM

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Artigo editado pelas normas da revista Marine and Freshwater Behaviour and Physiology

Observação: Tabelas e figuras foram mantidas no corpo do texto para facilitar a leitura da tese, além de um resumo em português.

Períodos cíclicos de restrição e realimentação no cultivo de *Litopenaeus vannamei* em sistema de bioflocos

Resumo

O presente estudo teve por objetivo a avaliação de diferentes períodos cíclicos de jejum e realimentação em um cultivo de camarões em sistema de bioflocos. Juvenis de *Litopenaeus vannamei* foram estocados a uma densidade de 300 camarões/m³. Cinco tratamentos foram testados: Controle, em que os animais foram alimentados diariamente; F5S2, 5 dias de alimentação seguidos por 2 dias sem receber ração, F3S4, 3 dias de alimentação seguidos por 4 dias sem receber ração, e F5S2-R e F3S4-R em que os dias de alimentação eram os mesmos citados acima, porém com redução das quantidades de alimento de acordo apenas com os dias em que eram alimentados. Foram monitorados a qualidade de água, o crescimento dos camarões e a comunidade microbiana nos tanques de cultivo. Ao final do estudo, os camarões dos tratamentos F5S2 e F5S2-R não apresentaram diferenças significativas comparados ao controle para sobrevivência, biomassa final, taxa de crescimento específico, taxa de conversão alimentar e produtividade. Os pesos finais similares entre o controle e F5S2 indicam crescimento compensatório completo, sendo justificado pelo aumento do consumo alimentar. Além disso, os resultados obtidos em F5S2-R indicam que a redução das taxas de arraçoameto além da quantidade de dias de alimentação (de 7 para 5 dias na semana) melhora a eficiência de conversão alimentar dos camarões no sistema de bioflocos.

Abstract

This study aimed to evaluate the use of different cyclical periods of starvation and re-feeding in a shrimp biofloc culture. *Litopenaeus vannamei* juveniles were stocked at 300 shrimp m^{-3}. Five treatments were tested: Control (fed intermittently); F5S2 (5 feeding days followed by 2 starvation days); F3S4 (3 feeding days followed by 4 starvation days). In these two treatments it was supplied the same amount of feed of the control group; F5S2-R and F3S4-R (the same feeding schedules but with reduced feed). Water quality, shrimp growth and microbial community were monitored. At the end of the study, the F5S2 and F5S2-R did not show significant differences among the control treatment for survival, final biomass, specific growth rate, food conversion ratio and productivities. Similar final weight between control and F5S2 indicate full compensatory growth, and this occurs due to increased feed intake. Furthermore, results of F5S2-R indicate that reducing feeding rates besides days of feeding improve the feed conversion efficiency, probably due to biofloc use as feed.

Keywords: biofloc, compensatory growth, shrimp culture, starvation, feeding management

1. Introduction

In intensive cultures, feeding has become a major management, and feeding strategy is an important practice that leads to growth, health, survival, and successful shrimp farming (Lin et al. 2012). The biofloc culture system is a technology that have been studied along the last decade, and the results obtained prove that it is possible to develop a system with low water use and better feed utilization when the natural productivity of the system is promoted and managed (Wasielesky et al. 2006; Krummenauer et al. 2014; Ray et al. 2010). Several studies about the contribution of natural productivity to shrimp nutrition have demonstrated that juvenile *L. vannamei* experience enhanced growth when cultured in the presence of microorganisms (Kent et al. 2011; Roy et al. 2012). However, according to Otoshi et al. (2011), differences in a number of parameters as water quality, feed quality, quantity and quality of natural productivity, stocking density, shrimp genetics and management, can influence the minor or major contribution to shrimp nutrition. When a “healthy” microbial community is developed in the system, it is possible to obtain greater results of survival and growth, reducing the amount of artificial feed to be offered and consequently, saving costs in production (Ray et al. 2010; Lara et al. 2016a).

Cyclical periods of food deprivation and re-feeding have been used as a management tool in order to obtain better growth results and save feed in aquaculture. One of the possible consequences of these cyclical feeding periods could be the compensatory growth (Wu and Dong 2002; Zhu et al. 2016). The possibility that, after a period of fasting the animals increase their food intake or their feed efficiency, leading them to reach same or larger size than the animals that experienced intermittent feeding can be considered an interesting strategy, compelling the animals to grow faster and make better use of the food provided and better use of the bioflocs as food source. In addition, reducing the number of days when the animals need to be fed can also generate a labor saving on farms.

Bioflocs are accumulation of algae, bacteria, protozoan and other kinds of particulate organic matter such as feces and unconsumed feed, which are held together in a matrix of mucus secreted by bacteria and bound by filamentous algae or held by electrostactic attraction (Hargreaves 2013). In the last years, the use of the biofloc system to maintain or promote accelerated growth in shrimp after an unfavorable period triggered by a particular factor (stocking density, temperature) has been used as a successful management tool, with animals achieving some degree of growth compensation (Wasielesky et al. 2013; Hostins et al. 2015; Fóes et al. 2016). Nevertheless, studies evaluating cyclical fasting and re-feeding periods, together with the reduction in quantities of food to be supplied are not conducted to know whether this microbial community in culture environment can be influenced by feed inputs as well as the influence of these variations on the growth of reared animals. In this way, the objective of the present study was to evaluate the effects of cyclical fasting and re-feeding periods on the growth parameters, water quality and microbial community in a shrimp biofloc culture system, with the potential reduction of the amount of artificial feed to be offered.

2. Materials and methods

2.1. Location and experimental design

The study was carried out at the Marine Station of Aquaculture, Federal University of Rio Grande (FURG), Southern Brazil. The experimental culture system was installed inside a greenhouse, in fifteen 180 L-tanks (150 L of useful volume). *Litopenaeus vannamei* juveniles (0.89 ± 0.35 g) were stocked at an initial density of 300 shrimp m^{-3} (n=45). The experiment was conducted over 42 days. Filtered marine seawater (sand filter), chlorinated at 10 ppm and dechlorinated with ascorbic acid (1ppm) was used to fill the tanks. An old biofloc from a culture that was in progress was utilized as inoculum (2.5% of the tanks volume, with a total suspended solids level of approximately 300 mg.L^{-1}) to start the biofloc formation according to the methodology proposed by Krummenauer et al. (2012). Organic fertilizations were performed according to Avnimelech (1999) and Ebeling et al. (2006) in order to maintain the C:N ratios between 15-20:1 and control the ammonia peaks. Commercial probiotic (Sanolife Pro-W, Inve Aquaculture) was applied according the manufacturer's specification every 3 days. There was no performed water exchange during the experimental period, and freshwater was added to the tanks to replace the levels lost by evaporation.

The treatment groups were defined according to different cycles of starvation and re-feeding, reducing or not the amount of artificial feed offered in these conditions. The proposed feeding schedule is detailed in Table 1. So, in the control group shrimp were fed continuously during all the experimental period, with a feeding rate corresponding to 100% of the calculation proposed by Garza de Yta et al. (2004). In the F5S2 treatment, shrimp were fed for 5 days and starved for 2 days in cyclical periods, and the amount of feed to be offered was the same of the control group, distributed into the fed days (7/7). In the F3S4, shrimp were fed for 3 days and starved for 4 days, in cyclical periods, and the amount of feed to be offered was the same of the control group, distributed into the fed days (7/7). In the F5S2-R treatment, shrimp were fed for 5 days and starved for 2 days in cyclical periods, and the amount of feed to be offered was reduced to only the days when the animals were fed (5/7). In the F3S4-R treatment, shrimp were fed for 3 days and starved for 4 days, in cyclical periods, and the amount of feed to be offered was reduced to only the days when the animals were fed (3/7).

Table 1 – Weekly feeding schedule utilized during the experimental period in different treatments.

Treatment	Feeding (days)	Starvation (days)	Quantity of feed in days a week	Quantity of feed (%)
Control	7	0	7/7	100%
F5S2	5	2	7/7	100%
F3S4	3	4	7/7	100%
F5S2-R	5	2	5/7	71.43%
F3S4-R	3	4	3/7	42.86%

2.2.Shrimp feeding and feeding parameters

Shrimp were fed two times a day with a commercial shrimp diet (Poti Active 38, 1.6 mm, D'Aguabi, Guabi Nutrição e Saúde Animal S.A., Campinas, São Paulo, Brazil). 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).

The shrimp were fed assuming a FCR of 1.45 and 1 g of growth per week. These values were calculated based on the mean values obtained in studies performed by Lara et al. (2016b; 2016c - submited) *(Capítulos 1 e 2 da presente tese)*. This expected weekly growth rate considers the animal genetics, stocking density, temperature and feed available in market currently in Brazil. Additionally, weekly, an estimated rate of

0.5% was reduced from the total number of stocked shrimps (estimated rate of natural mortality in the system) in each treatment in order to adjust the amount of feed supplied. Thus, the amount of feed to be offered was calculated according to the formulae as follows:

Feed (daily) = (Number of Shrimp x Expected Weekly Growth Rate x Expected FCR)/7.

Weekly, weighing (n=20) were performed in order to check the weekly growth rates, after the weighing all shrimp were returned to the respective treatment tank. At the end of the study, all animals from each tank were counted and weighted individually.

Additionally, the following parameters were calculated to evaluate shrimp growth and feeding parameters:

Specific growth rate in wet weight (SGR_w: % day^{-1}) = 100 x (lnW2 – lnW1)/t

Feed intake (FI: % body weight day^{-1}) = 100 x D_f / [t x (W1 + W2)/2]

Feed conversion efficiency in wet weight (FCE_w: %) = 100 x (W2 - W1)/D_f

Food conversion ratio (FCR) = (Final biomass – Initial biomass)/D_f

Productivity ($kg.m^{-3}$) = Final biomass (kg) – Initial biomass (kg)/volume of tank (m^3)

Where W2 and W1 – final and initial wet weight (g) of shrimp within a measure interval; t – the measuring interval (days); D_f – dry diet intake (g) within a measure interval.

2.3.Water quality monitoring

Daily, were monitored the temperature (°C), dissolved oxygen ($mg.L^{-1}$) and pH in tanks using a digital multiparameter (YSI Proplus, Yellow Springs, Ohio, USA). Weekly, were measured the total ammonia nitrogen ($mg.L^{-1}$) (UNESCO,1983), nitrite (mg.L^{-1}), nitrate (mg.L^{-1}), phosphate (mg.L^{-1}) (Strickland and Parsons, 1972), alkalinity (mg.L^{-1}) (APHA, 1989) and total suspended solids (mg.L^{-1}) (Strickland and Parsons, 1972). The alkalinity was corrected according to Furtado et al. (2011) using hydrated lime, maintaining the concentrations above 150 mg.L^{-1} during the study.

2.4. Microorganism analysis

At the end of the study, the main groups of microorganisms in each tank were observed and counted. The abundance of microorganisms was determined in 2.1-mL samples poured into sedimentation chambers (Utermöhl 1958) using an Olympus inverted light microscope equipped with phase contrast.

2.5. Statistical analysis

The results were analyzed to one-way ANOVA ($p < 0.05$). All tests were performed after confirmation of homogeneity of variances (Levene's Test) and data normality (Kolmogorov-Smirnov test). The Tukey test was applied to detect significant differences among treatments (Sokal and Rohlf, 1969).

3. Results

The water quality parameters analyzed during the experimental period are presented in Table 2. There were no observed significant differences among treatments for temperature (C°), dissolved oxygen (mg L^{-1}), pH, salinity, alkalinity (mg L^{-1}), total ammonia nitrogen (mg L^{-1}), nitrite (mg L^{-1}), nitrate (mg L^{-1}) and orthophosphate (mg L^{-1}). The only water quality parameter which presented significantly differences among treatments was the total suspended solids (mg L^{-1}). These values were lower in the F3S4-R treatment ($P < 0.05$) and did not show statistical differences among the other treatments ($P > 0.05$).

Table 2 – Water quality parameters monitored during the experimental period in different treatments.

Treatment/Parameter	Control	F5S2	F3S4	F5S2-R	F3S4-R
T (°C)	28.21 (±1.73)	27.47 (±1.70)	27.17 (±1.55)	28.08 (±2.14)	27.40 (±1.64)
D.O. (mg L^{-1})	6.22 (±0.53)	6.36 (±0.52)	6.34 (±0.54)	6.33 (±0.51)	6.48 (±0.51)
pH	8.23 (±0.20)	8.21 (±0.15)	8.19 (±0.16)	8.25 (±0.16)	8.31 (±0.15)
Salinity	26.00 (±3.69)	26.29 (±2.51)	25.95 (±3.17)	26.48 (±2.44)	25.89 (±2.40)
Alkalinity (mg $CaCO_3$ L^{-1})	153.77 (±28.38)	147.94 (±32.54)	162.17 (±35.10)	156.32 (±28.79)	155.96 (±24.05)
TSS (mg L^{-1})	473.33^b (±248.56)	365.00^b (±161.03)	344.69^b (±179.02)	373.00^b (±186.92)	271.25^a (±93.35)
N-AT (mg L^{-1})	0.24 (±0.51)	0.24 (±0.48)	0.26 (±0.66)	0.27 (±0.53)	0.18 (±0.27)
N-NO^-_2 (mg L^{-1})	0.43 (±0.89)	0.49 (±0.83)	0.58 (±1.64)	0.37 (±0.41)	0.29 (±0.28)
N-NO^-_3 (mg L^{-1})	16.50 (±13.71)	16.61 (±14.81)	21.00 (±15.69)	12.89 (±12.24)	10.89 (±11.02)
P-PO_4^{-3} (mg L^{-1})	3.05 (±1.96)	2.72 (±1.83)	3.35 (±2.14)	2.63 (±1.63)	2.20 (±1.38)

Values are means of replicates ± standard deviation. Different superscripts in the same row indicate significant differences ($P<0.05$).

The microbial community was composed mainly by centric and pennate diatoms. Particularly, the *Oocystis sp.* and *Nannochloropsis sp*. species were counted separately from other microalgae groups due to the great number of these microalgae present in the samples. Protozoan groups were defined as ciliates and flagellates, and some rotifers and nematodes also were observed in the water samples. The relative abundances of the main groups are showed in Table 3. The abundance of centric diatoms was lower in the F5S2 treatment ($P < 0.05$), followed by the F3S4 and F3S4-R treatments, that did not present differences between them ($P > 0.05$). The control and the F5S2-R treatments showed no differences between them in the abundance of this group ($P > 0.05$). The pennate diatoms were significantly lower ($P < 0.05$) in F5S2, F5S2-R and F3S4-R treatments, presenting no differences among them ($P > 0.05$). The abundance of *Oocystis sp.* was more dominant in the F3S4 treatment, presenting the higher densities ($P < 0,05$), followed by F5S2-R and F3S4-R, that did not present differences between them ($P > 0.05$). The lower densities of this specie were founded in control, followed by the F5S2 treatment; these treatments were significantly different between them for the abundance of this microorganism ($P < 0.05$). The microalgae *Nannochloropsis sp.* was observed in greater quantities in the F5S2-R treatment ($P < 0.05$) and the lower densities were registered in F5S2, F3S4 and the F3S4-R treatments, that did not present statistical differences among them ($P > 0.05$). This specie has an intermediate abundance in control treatment, and these densities were similar with the F3S4-R treatment. For ciliates and flagellates, the lower abundances of these two groups were recorded in F5S2 and F3S4 treatments ($P < 0.05$). Rotifers abundance was lower in F5S2-R and F3S4-R treatments ($P < 0.05$), followed by the F5S2 treatment. The higher densities of this microorganism were observed in control and F3S4 treatments which did not present significant differences between them ($P > 0.05$).

Nematodes were not observed in the F3S4 and F5S2-R treatments, and the higher abundance of this microorganism was registered in F3S4-R treatment ($P < 0.05$).

Table 3 – Mean abundance (organisms.10^{-6} mL^{-1}) of main groups of microorganisms identified in biofloc shrimp culture tanks.

Treatment	Centric diatom	Pennate diatom	*Oocystis sp.*	*Nannochloropsis sp.*	Ciliate	Flagellate	Rotifers	Nematodes
Control	0.73^{c} (±0.97)	0.85^{b} (±0.58)	2.70^{b} (±3.59)	1.23^{b} (±1.28)	0.68^{c} (±0.83)	0.91^{b} (±0.91)	0.14^{c} (±0.05)	0.02^{a} (±0.03)
F5S2	0.05^{a} (±0.05)	0.33^{a} (±0.10)	0.50^{a} (±0.16)	0.41^{a} (±0.28)	0.03^{a} (±0.01)	0.52^{ab} (±0.53)	0.06^{b} (0.02)	0.01^{a} (0.01)
F3S4	0.11^{b} (±0.14)	0.59^{b} (±0.13)	18.75^{d} (±25.9)	0.25^{a} (±0.35)	0.05^{a} (±0.03)	0.28^{a} (±0.19)	0.11^{c} (±0.09)	-
F5S2-R	0.51^{c} (±0.54)	0.48^{ab} (±0.17)	5.53^{c} (±6.26)	10.45^{c} (±13.54)	0.41^{c} (±0.45)	2.38^{c} (±2.38)	0.02^{a} (±0.02)	-
F3S4-R	0.14^{b} (±0.16)	0.25^{a} (±0.27)	7.12^{c} (±6.21)	0.53^{ab} (±0.76)	0.21^{b} (±0.01)	0.93^{b} (±0.80)	0.03^{a} (±0.01)	0.06^{b} (±0.03)

Values are means of replicates ± standard deviation. Different superscripts in the same column indicate significant differences ($P<0.05$).

The main growth and feeding parameters are demonstrated in Table 4 and the growth of shrimp over the 42 experimental days is showed in the Figure 1. The F3S4-R treatment presented the lowest final weight ($P < 0.05$). The final weigh in Control and the F5S2 treatment did not differ significantly between them ($P > 0.05$). Also, the F5S2, F3S4 and F5S2-R treatments did not show significant differences among them ($P > 0.05$). The survival was lower in F3S4 treatment ($P < 0.05$) and did not present differences among other treatments ($P > 0.05$). The weekly growth rates were lower only in F3S4-R treatment ($P < 0.05$) and were statistically equal in other treatments ($P > 0.05$). The specific growth rate was lower in F3S4-R ($P < 0.05$), and followed by F3S4 treatment that did not present significant differences among the F5S2 and F5S2-R treatments ($P > 0.05$) but differed from the control ($P < 0.05$). The control, in turn, did not differ from F5S2 and F5S2-R ($P > 0.05$) and was higher than F3S4 and F3S4-R treatments ($P < 0.05$).

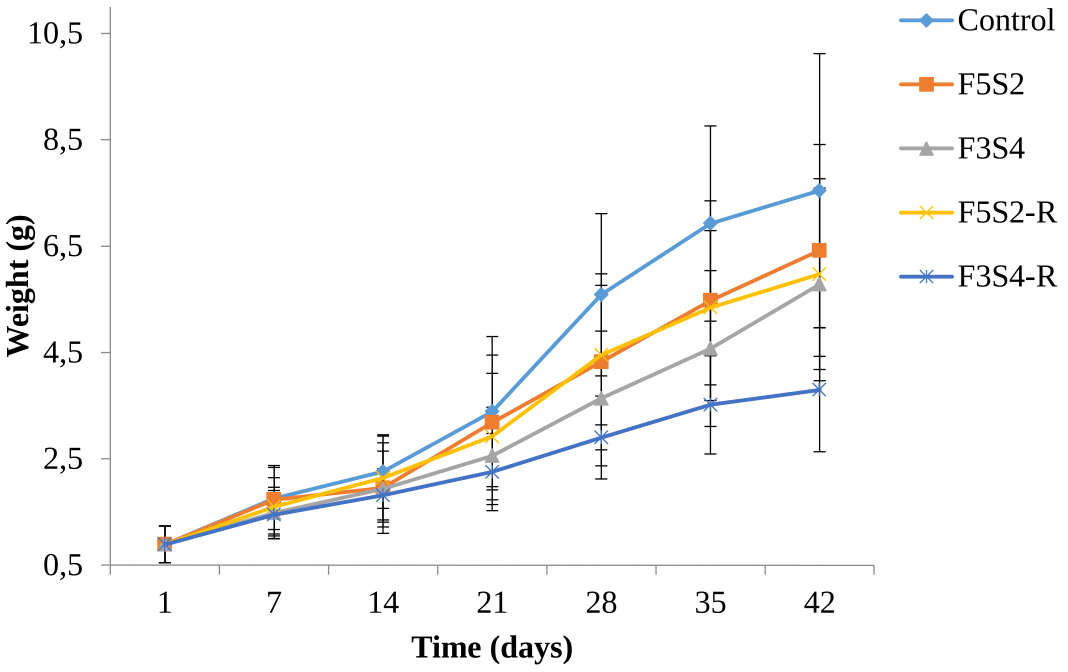

Figure 1 – Growth of *L. vannamei* over the experimental period.

Table 4 – Initial weight (W_i), final weight (W_f), survival (S%), final biomass (g), weekly growth rate (WGR – g week^{-1}), specific growth rate (SGR - % day^{-1}), feed intake (FI - % body weight day^{-1}), food conversion ratio (FCR), food conversion efficiency (FCE - %) and productivity (Prod. – kg m^{-3}).

	Control	F5S2	F3S4	F5S2-R	F3S4-R
W_i (g)	0.89 (±0.35)	0.89 (±0.35)	0.89 (±0.35)	0.89 (±0.35)	0.89 (±0.35)
W_f (g)	7.54^c (±2.58)	6.42^{bc} (±1.99)	5.78^b (±1.81)	5.97^b (±1.79)	3.82^a (±1.16)
S (%)	77.78^b (±15.56)	83.33^b (±11.00)	64.44^a (±4.44)	82.22^b (±8.01)	83.70^b (±14.80)
Final Biomass (g)	262.85^b (±46.84)	241.43^b (±43.08)	167.86^a (±19.44)	220.64^b (±15.23)	143.19^a (±20.32)
WGR	1.11^a (±0.62)	0.92^a (±0.36)	0.82^a (±0.30)	0.85^a (±0.36)	0.48^b (±0.15)
SGR	5.09^c (±3.15)	4.70^{bc} (±2.63)	4.45^b (±1.99)	4.53^{bc} (±2.46)	3.47^a (±2.21)
FI	5.85^b (±3.21)	6.51^c (±3.23)	8.05^d (±3.70)	4.99^b (±2.54)	3.86^a (±1.69)
FCR	1.31^a (±0.12)	1.48^a (±0.26)	2.31^b (±0.32)	1.23^a (±0.07)	1.34^a (±0.25)
FCE	75.37^{bc} (±49.64)	68.63^b (23.82)	42.00^a (±11.35)	80.94^c (±38.17)	76.49^{bc} (±26.79)
Prod (kg.m^{-3})	1.75^b (±0.31)	1.61^b (±0.29)	1.12^a (±0.13)	1.47^b (±0.10)	0.95^a (±0.14)

Values are means of replicates ± standard deviation. Different superscripts in the same row indicate significant differences ($P < 0.05$).

The feed intake (% body weight day^{-1}) was lower in F3S4-R ($P < 0.05$), followed by control and F5S2-R, that did not differ between them ($P > 0.05$). The feed intake in the F5S2 treatment was higher than control and F5S2-R treatments, and lower than the F3S4 treatment ($P < 0.05$). This treatment presented the highest feed intake compared to the other treatments ($P < 0.05$). The food conversion ratio was higher in the F3S4 treatment ($P < 0.05$) and did not differ statistically among other treatments ($P > 0.05$). Also, the food conversion efficiency was lower in F3S4 ($P < 0.05$), followed by F5S2 treatment. This treatment did not show significant differences among control and the F3S4-R treatments ($P > 0.05$). The F5S2-R treatment presented the better food conversion efficiency, and was not statistically different of the control and F3S4-R treatments ($P > 0.05$). The productivities achieved at the end of the study were lower in the F3S4 and the F3S4-R treatments ($P < 0.05$), and did not present differences among control, F5S2 and F5S2-R treatments ($P > 0.05$).

4. Discussion

The maintenance of suitable water quality parameters in biofloc culture system is totally dependent of the microbial community developed in tanks. Maintaining high aeration and mixing rates and strict control of the main water quality parameters allows achieving higher productivities at the end of the culture. The main temperature, dissolved oxygen, pH, salinity and alkalinity were maintained within the recommended levels for *L. vannamei*, did not influencing negatively the growth of animals (Van Wyk and Scarpa, 1999; Ponce-Palafox et al. 1997; Furtado et al. 2011; Maicá et al. 2012; Zhang et al. 2015a). Similarly, the main values of total ammonia nitrogen (TAN), nitrite ($N\text{-}NO^-_2$) and nitrate ($N\text{-}NO^-_3$) remain within the safety levels for the species and were not influenced by the different feeding strategies utilized (Lin and Chen, 2001; Lin and Chen, 2003; Furtado et al. 2015; Furtado et al. 2016).

The only parameter that was directly influenced by the treatments was the total suspended solids concentration. The treatment which received the small amount of feed at a frequency of 3 days of fed followed by 4 days of starvation, presented the lower values of TSS, indicating that possibly the shrimp graze upon the microbial community that composes the bioflocs. Additionally, the lower amount of artificial feed offered consequently leads to a reduction in organic matter present in the culture, potentially reducing the quantities of total suspended solids in water. Despite this difference in concentrations, the levels of total suspended solids remained within recommended levels for *L. vannamei* in biofloc system (Gaona et al. 2016).

The compensatory growth is a response in which animals are capable to recover their weight relative to the weight of an organism that has not experienced any period of stress (Jobling et al. 1994). These stressors factors could be due to a decrease in the amount, availability or quality of feed offered, or some environment culture condition, intentionally promoted or not, like temperature variations, low dissolved oxygen concentrations, high stocking density and different size of animals. In response to these periods, accelerated growth is observed and animals challenged could present different degrees of compensation. According to Ali et al. (2003), these degrees are differentiated into (i) full compensation, when deprived animals eventually achieve the same size at the same age as continuously fed contemporaries; (ii) partial compensation, when animals did not achieve the same size at the same age as nonrestricted contemporaries, but show increased growth rates, with improvement in food conversion ratios and; (iii) overcompensation, when animals achieve greater size at the same age that the nonrestricted group, showing a strong response in growth rates.

In present study, evaluating the growth of *L. vannamei*, it is possible to observe that both feeding frequency and the amount of feed offered influenced the final weight of shrimp. The F5S2 group presented full compensation when compared to the control treatment, and the other treatments presented partial compensation, did not achieving the same weight that the control after a 42-d period of cyclical starvation and re-feeding. In the study performed by Zhu et al. (2016) no significant differences were observed in final weight among shrimp fed continuously or with different feeding schedules mixing restriction and re-feeding periods. These authors indicate full compensatory growth of *L. vannamei* early juveniles (until reaching approximately 2 g) after 1, 2 or 3 days of fasting followed by re-feeding periods, in cyclical feeding schedules during 36 days.

Studies with different starvation and re-feeding cycles have been performed with different crustacean species in order to evaluate the changes in growth and feeding parameters to obtain compensatory growth and possibly save artificial feed (Pellegrino et al. 2008; Zhang et al. 2010; Rivera-Pérez and García-Carreño 2011; Zhang et al. 2015b). The SGRs are one of the parameters used to observe the growth response of animals after a stress period. In present study, these rates were negatively affected by the increase in periods of fasting and re-feeding in addition with the reduction in amount of feed offered. On the other hand, the treatments with 2 days of starvation followed by 5 days of re-feeding did not show differences for this parameter comparing with control, indicating that the reduction in days and in quantity of feed in this conditions did not affect the SGR, even if in the treatment with reduced quantity of feed the shrimp did not reach the same final weight.

Hyperphagia is by far the most common mechanism of growth compensation and can occur simultaneously or not with increased feed conversion efficiencies (Ali et al. 2003). Several studies reported these two mechanisms of compensation. Lin et al.

(2008) reported that the food intake was notably increased upon re-alimentation after 1-3 days of food deprivation in *L. vannamei*; Zhu et al. (2016) on the other hand, related increase in FCE in feed-restricted groups, suggesting that short term fasting had a potential improvement on the digestion and absorption function of white shrimp during re-feeding periods, resulting in compensatory growth. Similarly, Zhang et al. (2010), Wu and Dong (2001) and Stumpf and Greco (2015), related increased food intake and feed conversion efficiency, suggesting that these two mechanisms act simultaneously in achieve compensatory growth to *Feneropenaeus chinensis* and *Cherax quadricarinatus* under feed restriction, respectively. The F5S2 and F3S4 treatments presented increase in food intake when compared to the control, but did not show increased feed conversion efficiency, indicating that the compensatory growth observed, mainly in F5S2 treatment, occurred basically due to hyperphagia. In other restricted treatments, FCEs were similar to control, indicating that the potential compensatory responses observed occurred due to the increase in efficiency of feeding, but this increase was not sufficient to achieve the same final weight that the control. Wasielesky et al. (2013) did not evaluate the FCE and FI indexes, but relate compensatory growth of *L. vannamei* reared in biofloc culture system under different stocking densities in nursery phase when the animals were re-stocked at low stocking densities in grow-out phase. These authors concluded that BFT system can contribute to achieving full compensatory growth of animals. In present study, we can infer that the same beneficial effect of the culture system occurred.

When animals undergo a period of stress, their feeding behavior can be affected. Periods of food restriction in crustaceans are common, besides the variation in the availability of food in the natural environment, molting is a process which influences the food consumption. In premolt and before ecdysis stages, shrimp have their feeding declined and while the old cuticle is shed, the animals are unable to feed (Sanchez-Paz et al. 2007). In aquaculture systems, the molting process occurs in the same way that in natural environment, and the availability and quality of artificial and natural feeds in tanks or ponds represent an important point that deserves attention from the farmers in order to achieve better growth and save production costs. The lower survival observed in treatment F3S4, when compared to the treatment F3S4-R could be due the cannibalism that probably occurs during molting process. According to Comoglio et al. (2004), *L. vannamei* exposed to periods of starvation presents an adaptive strategy in which stop spending its energy in molting in order to save their reserves. Thus, the shrimp maintain their body weight and did not suffer the risk of cannibalism and mortality in the molting. This probably occurred in F3S4-R treatment, in which shrimp did not presented high weight increase but their survival was similar to the control treatment.

According to Ray et al. (2010), recognizing the occurrence of some potentially harmful (i.e. cyanobacteria) or beneficial (i.e. diatoms) groups of microorganisms in cultures allow to take measures to prevent risks or improve the use of the natural productivity of the system. Regarding to the microbial community observed in the present study, the microorganisms are those that normally appear on biofloc cultures and this contribution is variable according to the main abundance in culture environment (Ray et al. 2010; Vinatea et al. 2010; Suita et al. 2015; Lara et al. 2016a). Particularly, the two groups of Chlorophyceae observed in great abundance in treatments, *Oocystis sp.* and *Nannochloropsis sp.*, are species that have been observed in biofloc cultures. Kent et al. (2011), indicated that consumption of *Nannochloropsis* by *L. vannamei* was low and variable, and suggests that although shrimp juveniles (mean weight 2 g) can ingest this genus of microalgae they are unable to digest them. In

F3S4 treatment, the relative abundance of *Oocystis sp.* observed at the end of the study was higher than other treatments and, coincidently, the survival observed was lower. On the other hand, none of other parameters analyzed seems to have affected negatively the shrimp survival and FCR, which may be evidence that the presence of greater abundances of these microalgae in superintensive systems could cause any loss in production, even though there are no studies indicating that this genus could influence the survival of shrimp.

According to Decamp et al. (2001) ciliates and flagellates could represent an important source of highly unsaturated fatty acids (HUFAs) and steroids, and their intracellular content is rich in free amino acids, contributing to shrimp growth. This growth effect has been reported by another authors that detected the presence of these groups of microorganisms in biofloc systems (Lara et al. 2016). The control group was the treatment which showed larger abundances in diatoms, ciliates, flagellates and rotifers, followed by the F5S2-R, that presented similar abundances that in control treatment. When relating this increased abundances and diversities for both treatments with the shrimp performance, it may possible to suggest that this characteristic of microbial community contribute to the better productivities observed.

Overall, when analyzing the final biomass (g) and the productivities obtained at the end of the study and considering the other parameters, it is possible to conclude that the increase in starvation period from 2 to 4 days in cyclical periods affected negatively the shrimp growth. On the other hand, using a feeding schedule of 2 days of starvation followed by 5 days of re-feeding with reduced feeding rates it is possible to obtain the same productivities that in non-restricted treatments. These results indicate that the biofloc culture system allows conditions in that are possible to obtain high yields, reducing even the manpower as the amount of food to be offered to reared animals. This reduction contributes to the system to be more cost-effective, representing important cost saving in terms of farming.

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CAPÍTULO 4

THE USE OF BIOFILM AND DIFFERENT FEEDING RATES IN BIOFLOC CULTURE SYSTEM

Running head: Biofilm and feeding rates in shrimp biofloc culture

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Artigo submetido à revista Aquaculture International

Observação: Tabelas e figuras foram incluídas no corpo do texto para facilitar a leitura da tese, além de um resumo em português.

Utilização de biofilme e diferentes taxas de arraçoamento no cultivo em sistema de bioflocos: Efeitos no desempenho zootécnico dos camarões

Resumo

A utilização da tecnologia de bioflocos juntamente com biofilme pode beneficiar o cultivo de camarões através da redução da quantidade de alimento adicionado ao sistema. Este estudo avaliou a utilização de biofilme no cultivo de *Litopenaeus vannamei* em sistema de bioflocos com diferentes taxas de arraçoamento. Juvenis (0,89 ± 0,35 g) foram estocados a uma densidade de 300 camarões/m³ em 24 tanques de 150L. As taxas de arraçoamento foram calculadas considerando uma taxa de crescimento semanal esperada de 1 g/semana e uma mortalidade estimada de 0,5%/semana. Cada tratamento correspondeu a uma taxa de arraçoamento diferente e cada uma dessas taxas correspondeu a uma taxa de conversão alimentar fixa. Assim, os tratamentos testados foram: T0 e T0+B (sem adição de ração, com e sem biofilme, respectivamente), T0.6 e T0.6+B, T1.2 e T1.2+B, T1.8 e T1.8+B. O estudo teve duração de 42 dias. Ao final do experimento, os camarões que foram cultivados sem adição de ração apresentaram os menores pesos finais e sobrevivências, independente da utilização de biofilme. O tratamento T1.2+B não apresentou diferenças significativas entre T1.2, T1.8 e T1.8+B para os parâmetros de crescimento e alimentação. As sobrevivências foram acima de 91% em todos os tratamentos que receberam ração e não foram detectadas diferenças significativas entre esses tratamentos ($P > 0.05$). Os resultados permitem concluir que a presença de biofilme no tratamento T1.2+B representou uma economia de 35% do total de ração a ser oferecido. Isso pode representar um valor significante em termos de custos de operação, melhorando a sustentabilidade do sistema de bioflocos.

ABSTRACT

The use of bioflocs technology in conjunction with biofilm can benefit shrimp farming by reducing the amount of feed supplied. This study evaluated the use of biofilm in a *Litopenaeus vannamei* biofloc system using different feeding rates. Shrimp juveniles (0.89 ± 0.35 g) were stocked at 300 shrimp m^{-3} in 24 150-L tanks. The feeding rates were calculated by 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 FCR. Thus, the treatments tested were: T0 and T0+B (without addition of artificial food, with and without biofilm addition, respectively); T0.6 and T0.6+B; T1.2 and T1.2+B; T1.8 and T1.8+B. The study lasted 42 days. At the end of the study, shrimp that were grown with no artificial food presented lower final weights and minor survival, independent of the addition of biofilm. The T1.2+B treatment did not differ significantly from the T1.2, T1.8 and T1.8+B treatments for the growth and feeding parameters. The survivals were higher than 91% in all of the feed treatments, and no significant differences were detected among these treatments. In contrast, the results allowed the conclusion that the presence of biofilm in the T1.2+B treatment represented a feed saving of 35% of the total amount of artificial food offered. This could represent a significant value in the cost of operation and may make the BFT system more cost-effective and environmentally friendly.

Keywords: artificial substrates; biofloc system; feeding; management; natural productivity; shrimp culture.

INTRODUCTION

Shrimp farming in a biofloc system is a technology that has been successfully employed by many producers who want to make their cultures more environmentally sustainable. Through the formation of a microbial community by elevating the C: N ratio in water, the water quality of the culture can be improved. The nutrients that are discharged can be recycled into the culture environment, and these microorganisms can be used as a supplementary food for the grown animals (Wasielesky *et al*. 2006; Krummenauer *et al*. 2014; Martínez-Córdova *et al*. 2014; Khanjani *et al*. 2016). The bioflocs are composed from a wide range of microorganisms, such as heterotrophic and autotrophic bacteria, microalgae, protozoans, rotifers and nematodes, and this microbial community can be adhered, forming aggregates, or be freely suspended in the water (Ray *et al*. 2010; Lara *et al*. 2016). The nutritional composition of these aggregates can vary, but the availability of natural productivity in the culture tanks 24 hours per day represents an important supplementary food source to the cultured animals, enhancing growth parameters and, immunological responses (Xu and Pan 2012; Ekasari *et al*. 2014).

In addition, many studies with different species reported that the addition of substrates (biofilm) in culture tanks is a technique that perceptibly improves the water quality, mainly reducing the concentrations of nitrogen compounds and phosphate (Thompson *et al*. 2002; Viau *et al*. 2013; Shilta *et al.* 2016). For biofilms used directly as a food source, Abreu *et al*. (2007) observed that microorganisms present in biofilm may contribute to *Farfantepenaeus paulensis* growth of over 49% of carbon and 70% of nitrogen necessities. Studies addressing the importance of biofilm to *Litopenaeus vannamei* culture proved that the substrates could also be an important food source to the animals, improving the productivities through better survival rates, reducing the need for artificial feed and, consequently, reducing the food conversion ratios (Audelo-Naranjo *et al*. 2011; Huang *et al*. 2013; Zhang *et al*. 2016).

The use of these two cited techniques together in farming may further improve the viability of shrimp cultures because their benefits together may lead to a more sustainable aquaculture through greater utilization of natural food and increased stocking densities. In a study with *Penaeus monodon* juveniles, Arnold *et al*. (2009) demonstrated that the growth of the shrimp could be significantly enhanced through the use of high intensity microbial floc with the addition of artificial substrates. In another study, Schweitzer *et al*. (2013) verified that the substrates served to increase the surface area to *L. vannamei*, and the use of biofilm in a biofloc culture allowed a more intensified system, resulting in higher production indices. In the results obtained by Zhang *et al*. (2016), it was indicated that the utilization of artificial substrates in a zero water exchange culture system could effectively control the water quality, improve the survival and growth of shrimp and significantly reduce the FCR in winter conditions.

According to Nevejan *et al*. (2016), many crustacean species are considered omnivorous in at least some life stages and consume detritus, flocculated organic material and biofilm. In the adult stage, most farmed crustaceans have a raptorial feeding behavior, feeding on larger feed particles, which they manipulate and fragment outside of their bodies prior to ingestion. Despite knowing that adult crustaceans do not have filter feeding capacities, it was proven that *L. vannamei* could obtain nutritional benefits from bioflocs and/or biofilm (larger particles in form of aggregates), and this capacity was related to the morphological structure of the third pair of maxillipeds (Kent *et al*. 2011; Kim *et al*. 2015). In this context, adding the possible beneficial nutritional effects of aggregates in suspension (bioflocs) adhered to substrates (biofilm or periphyton), it is expected that there would be a reduction in the amount of artificial food to be supplied, or at least an improvement in feed efficiency in the animals grown.

Therefore, the objective of this study was to evaluate the use of artificial substrates in a biofloc culture system, using different feeding rates in order to verify whether or not the presence of an extra source of natural food (biofilm) could reduce the amount of feed provided in a superintensive culture of *L. vannamei*, isolating the possible effects of water quality on shrimp growth.

MATERIAL AND METHODS

Location:

The study was carried out at the Marine Station of Aquaculture, Institute of Oceanography, Federal University of Rio Grande, Southern Brazil.

Experimental design and feeding rates:

L. vannamei juveniles (initial weight: 0.89 ± 0.35 g) were stocked in triplicate tanks at an initial stocking density of 300 shrimp/m³ (n=45). The experiment was carried out for 42 experimental days.

Three different feeding rates and one treatment in total absence of artificial food, with and without the presence of artificial substrates to biofilm attachment, were tested. Thus, T0 and T0+B were the treatments without the addition of artificial feed, and the T0.6 and T0.6+B, T1.2 and T1.2+B and T1.8 and T1.8+B were the feed treatments; each one corresponded to a different fixed FCR.

A water biofloc recirculation system was designed to isolate the effects of different feeding rates and the biofilm addition on the water quality. This system was proposed to only observe the effects of different feeding rates and the contribution of the bioflocs on the shrimp performance. The system contained 24 150-L tanks (microcosm tanks), with a bottom area of 0.5 m² and individual aeration. All of the tanks had water output driven by gravity into a return pipe, which led to the matrix 20 m³-tank. In this matrix tank, an inoculum of 10% of old biofloc was used to promote the 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 with the aid of a pump, with a flow rate of 2.08 L/min per tank (20 exchanges per day). The water quality in the matrix and microcosm tanks was monitored prior to initiating the study, in order to observe if the concentrations of the main factors were maintained in the same values in these units.

The artificial substrates (Needlona) non-floating nets (mesh size of 1.0 mm) were placed vertically in the units, and the surface was submerged during the entire study period. An increase in the total surface of 150% was calculated based on the bottom area of the microcosm tanks (0.5 m²). The structures were submerged two weeks prior to the start of the study for biofilm colonization, and the recirculating biofloc system was put into operation without shrimp in the microcosm tanks during these days.

The daily feeding rates were calculated based on the methodology proposed by Garza de Yta *et al*. (2004). The feeding rates were calculated by assuming different FCRs and 1g of growth per week. Each different FCR was assumed to correspond to a different feeding rate. The formula for the calculation of daily feeding was as follows:

Feed (daily) = (Number of Shrimp x Expected Weekly Growth Rate x Expected FCR)/7.

Shrimp were fed a 38% protein commercial shrimp diet (Poti Active 38, 1.6mm, D’Aguabi, Guabi Nutrição e Saúde Animal S.A., Campinas, São Paulo, Brazil). 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).

The feed was offered two times a day via feeding trays. Uneaten feed was removed from the feed trays every morning and dried in an oven (Biopar Equipamentos Eletro-Eletrônicos Ltda., Model S150SD2) at 60°C until constant weight. After this, the final dry weight was recorded using a digital balance with 0.001g of precision (Shimadzu, Model UX420H). Feed leaching and percent moisture of the artificial feed were measured prior to the start of the study in order to determine the feed stability.

Calculation of data:

Weekly weighing (n=20) was performed in order to check the weekly growth rates. At the end of the study, all of the animals from each tank were counted and weighed individually.

Culture monitoring:

The temperature (°C), dissolved oxygen (mg.L^{-1}) and pH in the microcosm and macrocosm tanks were monitored daily using a digital multiparameter (YSI Proplus, Yellow Springs, Ohio, USA). The total ammonia (UNESCO 1983), nitrite, nitrate, phosphate (Strickland and Parsons, 1972), alkalinity (APHA 1989) and total suspended solids (Strickland and Parsons 1972) from the matrix tank were measured weekly.

In the matrix tank, organic fertilizations with molasses were conducted in order to maintain a C:N relationship of approximately 15-20:1. These fertilizations followed the methodologies proposed by Avnimelech (1999) and Ebeling *et al*. (2006). Commercial probiotic (Sanolife Pro-W, Inve Aquaculture) was applied according the manufacturer’s specification every 3 days. Similarly, the alkalinity was corrected according to Furtado et al. (2011) using hydrated lime to maintain the concentrations above 150 mg.L^{-1} during the study.

Statistical analysis:

The results were analyzed by a one-way ANOVA ($p < 0.05$). All of the tests were performed after confirmation of homogeneity of variances (Levene's Test) and data normality (Kolmogorov-Smirnov test). The Tukey test was applied to detect significant differences among treatments (Sokal and Rohlf 1969).

RESULTS

In table 1 are presented the mean values of temperature (°C), dissolved oxygen concentration ($mg.L^{-1}$) and pH registered in microcosm tanks over the 42 experimental days. These parameters did not present significant differences among treatments ($P>0.05$). In the macrocosm tank, the mean total ammonia nitrogen (TAN) concentration was 0.08 (±0.07) $mg\ L^{-1}$, the nitrite-nitrogen ($N\text{-}NO^{-}_{2}$) was 0.09 (±0.05) $mg\ L^{-1}$, the nitrate-nitrogen ($N\text{-}NO^{-}_{3}$) was 15.17 (±8.52) $mg\ L^{-1}$, the orthophosphate ($P\text{-}PO_4^{-3}$) was 2.06 (±1.05) $mg\ L^{-1}$, alkalinity was 158.82 (±27.12) $mg.L^{-1}$, salinity 25.50 (±2.25) and the mean total suspended solids (TSS) were 181.25 (±107.09) $mg.L^{-1}$.

Table 1 – Water quality parameters (means ± standard deviation) monitored daily in the microcosm tanks during the 42 days of the study.

Treatment/ Parameter	T (°C)	O.D. (mg L^{-1})	pH
T0	28.88 (±0.58)	6.21 (±0.44)	8.22 (±0.10)
T0+B	28.89 (±0.60)	6.14 (±0.45)	8.21 (±0.10)
T0.6	28.99 (±0.52)	6.14 (±0.53)	8.20 (±0.10)
T0.6+B	28.93 (±0.55)	6.07 (±0.44)	8.20 (±0.10)
T1.2	28.95 (±0.56)	6.05 (±0.46)	8.20 (±0.11)
T1.2+B	29.05 (±0.55)	5.98 (±0.47)	8.18 (±0.10)
T1.8	29.08 (±0.59)	6.08 (±0.49)	8.20 (±0.10)
T1.8+B	28.96 (±0.55)	6.10 (±0.46)	8.19 (±0.09)

The mean growth, feed and feeding parameters of shrimp are presented in Table 2. The final weights presented higher values with the increase in feeding rates. In figure 1 is presented the growth of shrimp over the experimental period. At the end of the study, the treatments that did not receive any artificial food presented the lowest final weights ($P < 0.05$), and the biofilm did not affect this parameter between these two treatments. Similarly, there were no observed significant differences in the final weights between the T0.6 and T0.6+B treatments, T1.2 and T1.2+B and T1.8 and T1.8+B ($P > 0.05$). Also, there were no observed significant differences ($P > 0.05$) among treatments T1.2+B, T1.8 and T1.8+B.

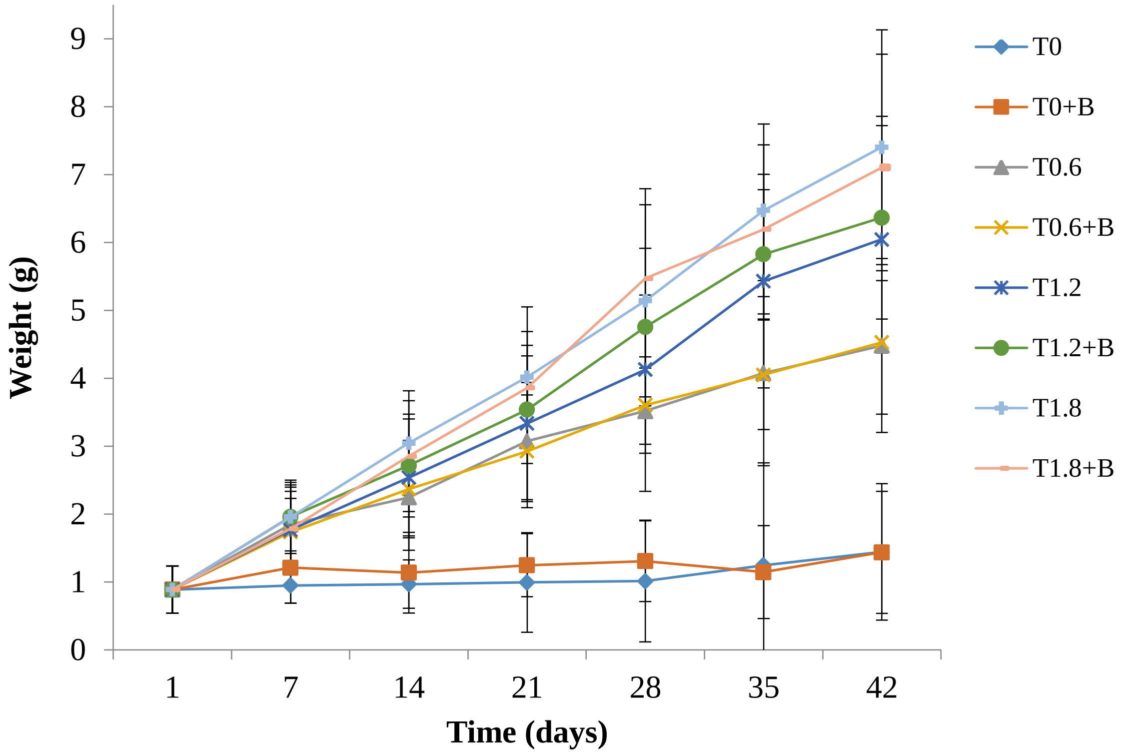

Figure 1 – Growth of *L. vannamei* during the 42 experimental days in biofloc culture with and without addition of artificial substrates at different feeding rates.

In table 2 are showed the mean growth and feeding parameters monitored along the study. The weekly growth rates (WGR) followed the same pattern of increase with the increasing in feeding rates. The use of biofilm did not influence the WGRs between the treatments with the same amount of artificial food. The treatments with fixed FCRs of 1.2 presented intermediate values of WGRs and were statistically similar to the T0.6 and T1.8 treatments, independent of the biofilm presence. The SGR (% day^{-1}) was lower in the T0 treatments ($P < 0.05$), followed by the T0.6 treatments ($P < 0.05$), and the biofilm did not influence this parameter. The T1.2 and T1.8 treatments did not present differences between them, and in the same way, the SGR was not influenced by the addition of biofilm in tanks ($P > 0.05$). The final FCRs obtained were lower in the T0.6 and T0.6+B treatments ($P < 0.05$), followed by T1.2, which did not differ significantly from the two higher feeding rates (T1.8 and T1.8+B) ($P > 0.05$), and the T1.2+B were similar only to their respective feeding rate ($P > 0.05$). The food conversion efficiency (%) was higher in the T0.6 treatments ($P < 0.05$), independent of the addition of artificial substrates. The T1.2 treatment presented an FCE similar to the T1.8 treatments ($P > 0.05$) and did not present differences between the T1.2+B treatment ($P > 0.05$).

The survivals were lower in the T0 and T0+B treatments ($P < 0.05$), and the addition of biofilm did not influence this parameter. In all of the other treatments, the recorded survivals were above 91% and did not present significant differences among them ($P > 0.05$).

The productivities achieved at the end of the study were lower in T0, independent of the biofilm addition ($P < 0.05$), followed by the T0.6 treatments. The T1.2 treatment presented intermediate productivity but was lower than the T1.8 treatments ($P < 0.05$). Also, the T1.2+B treatment did not differ significantly from the T1.8 and T1.8+B treatments ($P > 0.05$).

Table 2 – Mean initial and final weight (g), survival (%), weekly growth rates (g week^{-1}), specific growth rates (% day^{-1}), food conversion ratios, feeding conversion efficiency (%) and productivities (kg.m^{-3}) registered during the 42 experimental days.

Treatment	Weight$_{\text{Initial}}$	Weight$_{\text{Final}}$	Survival (%)	WGR	SGR	FCR	FCE	Productivity
T0	0.89 (±0.35)	1.44^{a} (±1.00)	28.15^{a} (±6.79)	0.09^{a} (±0.10)	1.11^{a} (±2.87)	-	-	0.12^{a} (±0.02)
T0+B	0.89 (±0.35)	1.44^{a} (±0.90)	34.07^{a} (±22.48)	0.09^{a} (±0.19)	1.12^{a} (±3.18)	-	-	0.14^{a} (±0.07)
T0.6	0.89 (±0.35)	4.48^{b} (±1.28)	91.85^{b} (±10.50)	0.60^{b} (±0.24)	3.85^{b} (±3.26)	0.86^{a} (±0.05)	120.84^{c} (±67.92)	1.23^{b} (±0.06)
T0.6+B	0.89 (±0.35)	4.53^{b} (±1.06)	98.52^{b} (±2.57)	0.61^{b} (±0.15)	3.88^{b} (±3.07)	0.77^{a} (±0.03)	135.47^{c} (±74.96)	1.34^{b} (±0.05)
T1.2	0.89 (±0.35)	6.05^{c} (±1.68)	92.59^{b} (±3.39)	0.86^{bc} (±0.23)	4.56^{c} (±2.92)	1.35^{bc} (±0.08)	75.96^{ab} (±31.14)	1.68^{c} (±0.09)
T1.2+B	0.89 (±0.35)	6.37^{cd} (±1.49)	97.78^{b} (±2.22)	0.91^{bc} (±0.25)	4.69^{c} (±3.42)	1.19^{b} (±0.05)	86.53^{b} (±38.00)	1.87^{cd} (±0.07)
T1.8	0.89 (±0.35)	7.40^{e} (±1.73)	94.07^{b} (±6.42)	1.09^{c} (±0.14)	5.04^{c} (±3.25)	1.63^{c} (±0.24)	63.67^{a} (±19.37)	2.09^{d} (±0.25)
T1.8+B	0.89 (±0.35)	7.11^{de} (±1.67)	99.26^{b} (±1.28)	1.04^{c} (±0.31)	4.95^{c} (±3.06)	1.58^{c} (±0.03)	65.46^{a} (±28.28)	2.12^{d} (±0.05)

Values are means of replicates ± standard deviation. Different superscripts in the same row indicate significant differences ($P<0.05$).

DISCUSSION

Studies using biofloc recirculation systems have been conducted in order to isolate the possible effects (positive or negative) on water quality and the amount of natural productivity in each experimental unit. The experimental design showed efficiency in separating the effects of different feeding rates and the addition of artificial substrates in shrimp culture. The mean temperature, dissolved oxygen and pH recorded in the microcosm tanks were maintained within the levels recommended for *L. vannamei* (Ponce-Palafox *et al.* 1997; Van Wyk and Scarpa 1999; Furtado *et al.* 2011; Zhang *et al.* 2015). Similarly, all of the water quality parameters monitored in the macrocosm tank (alkalinity, salinity, total ammonia nitrogen, nitrite, nitrate, orthophosphate and total suspended solids) were kept at the optimum concentrations for shrimp culture, and they did not negatively affect the food consumption and growth of the animals (Lin and Chen 2001; Lin and Chen 2003; Furtado *et al.* 2011; Maicá *et al.* 2012; Furtado *et al.* 2015; Gaona *et al.* 2015).

Many authors reported that the addition of biofilm significantly improved the growth and survival of different species through improved water quality. Viau *et al.* (2012), in a study of *Cherax quadricarinatus*, related that higher survival was associated with a better water quality maintained by biofilm, in terms of low levels of both ammonia and nitrite, together with high levels of pH and dissolved oxygen. Thompson *et al.* (2002) found better water quality in the culture of *Farfantepenaeus paulensis* with increased weight and total biomass of the shrimp juveniles. In recent studies with *L. vannamei*, Huang *et al.* (2013) and Zhang *et al.* (2016) stated that artificial substrates contributed to the growth rate of juveniles through the improvement of water quality in terms of reduced total ammonia and nitrite nitrogen compounds. Also, Ferreira *et al.* (2016) combined the use of biofilm with biofloc culture system and observed that the artificial substrates possibly contributed to the control of excess suspended solids in the water, which consequently led to better final weights of *L. vannamei*. In the present study, because the biofloc recirculation system did not allow the water quality to be influenced by the addition of artificial substrates, it was possible to separately observe the effects that the biofilm combined with the biofloc culture system and the different amounts of food offered exerted on the shrimp growth performance.

The biofilm addition was not always related to the increased performance of cultured animals. Viau *et al*. (2013) studied the use of artificial substrates in *Farfantepenaeus brasiliensis* nursery and did not observe differences in either mean body weight or weight gain between the use or non-use of artificial substrates in a periphyton-based zero water exchange culture system. In the same way, Kumlu *et al*. (2001) demonstrated that the use of either vertically or horizontally placed substrates did not provide any advantage during the nursery culture of *Metapenaeus monoceros*. On the contrary, there have been many studies that reported that the use of biofilm could contribute to reduced feeding costs, through the reduction of protein and/or vitamin and mineral supplementation in artificial diets (Ballester *et al*. 2007), reduction in the amount of food added (Audelo-Naranjo *et al*. 2011) or even in the total absence of artificial feed (Audelo-Naranjo *et al.* 2012). In the present work, the use of artificial substrates did not influence the final weights of shrimp within treatments with the same feeding rates, but the non-significant differences in the main growth parameters among the T1.2+B treatment and the T1.8 and T.8+B treatments represent a possibility to reduce the feed amounts using biofilm as a supplemental feed source.

Moss and Moss (2004) showed a significant reduction in the negative effects of stocking density when using artificial substrates in an *L. vannamei* nursery. The same authors recommended separating the effects of shrimp behavior, water quality, pond bottom conditions and food availability to observe these negative effects. The non-addition of artificial feed significantly compromised the survival of animals, independent of the artificial substrates addition, indicating that food availability was a key factor that led to poor results in these treatments. These results were not in agreement with studies of Abreu *et al*. (2007), which observed a survival of 90.5% in a 30-day study of *F. paulensis* grow-out without the addition of ration, and Audelo-Naranjo *et al*. (2012), which grew *L. vannamei* in the total absence of artificial feed for 40 days and observed survivals between 90.7 % and 97.3% using only a biofilm food source in a zero water exchange system. In spite of these better results, it is important to highlight that the stocking densities utilized in both studies were much lower than that used in the present study (20 shrimp.m^{-2} in the first, and a maximum of 32 shrimp.m^{-2} in the second study). A significant reduction in survival could be observed when no food was supplied to *L. vannamei* in the study of Voltolina *et al*. (2013), which had survivals near 60% using only artificial substrates to feed shrimp juveniles for 38 days.

On the contrary, systems which combined the biofloc and biofilm technologies demonstrated increased survival rates. Schveitzer *et al*. (2013) observed significantly higher survival in tanks with substrates (93.9 ± 2.4), and Ferreira *et al*. (2016) obtained survivals over 85.6% using artificial substrates in a BFT system. Both studies utilized stocking densities similar to the present study, indicating that the addition of biofilm could be a good tool to improve the survival in the grow-out of *L. vannamei* in this culture system. Another important observation relative to the survival in feed treatments is that, even with lower feeding rates (T0.6), the survivals were high (above 90%), showing that shrimp could be reared in the BFT+biofilm system with a small amount of ration without negatively compromising their survival.

The microorganisms present in these structures could represent an important food source to the reared animals that graze upon these communities as a selective feeding strategy, reducing feeding costs and being an environmental friendly alternative (Silva *et al*. 2008). The biofloc technology, in turn, could equally contribute to reduce the amount of feed offered to cultured animals, improving the FCRs observed through the rearing period (Wasielesky *et al*. 2006). In the present study, presumably, the higher FCRs were observed in T1.8 and in the T1.2 treatments, without biofilm. In the presence of biofilm, the T1.2 feeding rate showed a better FCR at the end of the study, proving that it was possible to reduce the feed amounts utilizing the artificial substrates. Calculating this saving in terms of quantity of food added, it is estimated that approximately 35% less feed can be offered to shrimp if natural productivity provided by the artificial substrates is present in the biofloc culture system. The FCR values obtained were very similar to the values observed by Huang *et al*. (2013), and Zhang *et al*. (2016) used artificial substrates in stocking densities of 150 shrimp.m^{-2} and 250 shrimp.m^{-3}. Better FCRs (0.96 and 0.97) were presented by Ferreira *et al*. (2016) at same stocking density (300 shrimp.m^{-3}) but with a larger increase in the number of substrates (200% and 400% of the lateral area of tanks). Schveitzer *et al.* (2013) observed mean FCRs of 1.6 and 1.7 in stocking densities of 238 shrimp.m^{-3} and 473 shrimp.m^{-3}, respectively, which could be considered higher results than those observed in the present study.

The SGRs (%.day^{-1}) did not show significant improvement among treatments fed with 1.2 and 1.8 fixed FCRs. Zhang *et al*. (2016) did not observe an increase in SGR when artificial substrates were used in a 10-week *L. vannamei* grow-out. Ferreira *et al*.

(2016) already demonstrated that this parameter was significantly increased with the substrate addition, but for a period of 35 days, with shrimp growing in a size range from 0.40 g to 3.43 g, which could be considered a size range in which shrimp could graze in the substrates more efficiently than larger shrimp. Likewise, the SGRs observed in the studies cited above were very similar to the values observed in the present study.

The WGRs observed in the T0 treatments were very similar to the results found by Voltolina *et al*. (2013) in the absence of artificial feed, but in semi-intensive density condition. Studies reported that the WGR was another parameter that was positively influenced by the substrate inclusion in culture (Audelo-Naranjo *et al*. 2011; Schveitzer *et al*. 2013). In the present study, even though there were no observed significant differences within the feed treatments with the same feeding rates, two important observations are important to highlight: (i) the T0.6 treatments, although it presented weekly growth rates lower than T1.2 and T1.8 treatments, showed good growth results (around 0.6 $g.week^{-1}$); and (ii) the increase in feeding rates from 1.2 to 1.8 fixed FCRs did not influence the weekly growth rates in these treatments, and the artificial substrates did not represent a factor that interfered in this parameter in the conditions proposed by the experimental design.

The feed conversion efficiency (FCE %) is a parameter utilized in order to verify if the animals are converting the feed added in the system more efficiently due to some special factor. A decrease in these values could be related to over-feeding in aquaculture systems and associated to food waste, affecting the profitability of the culture. No reports in the literature showed that the use of biofilm could improve these rates, even knowing that the additional food provided by the biofilm could contribute to the reduction in the quantities of feed and better incorporation of C and N, due to increased recycling nutrients in the cropping system (Abreu *et al*. 2007; Audelo-Naranjo *et al*.

2012). By analyzing the results of the present study, in general, the treatments with artificial substrates presented FCEs approximately 10% higher than treatments without the addition of biofilm, indicating that the inclusion of these structures could contribute to better feeding efficiency.

The final productivities achieved followed the same pattern observed for all of the other major growth parameters. No significant differences were observed among the T1.2+B treatment and the T1.8 and T1.8+B treatments, showing that no extra food was necessary to obtain the same productivities when using BFT+biofilm consortium. Similarly, these values were very close to those found by Audelo-Naranjo *et al.* (2011), which obtained productivities around 2 $kg.m^{-2}$ with similar stocking densities in *L. vannamei* culture. These values were lower than the productivities reported by Schveitzer et al. (2013) that found values of 2.9 $kg.m^{-3}$ with substrate addition. These values were higher than those observed by Zhang *et al.* (2016), which reported a productivity of 0.93 $kg.m^{-3}$ with biofilm addition.

In conclusion, at high stocking densities (> 300 $shrimp.m^{-3}$), using the biofloc and biofilm techniques together was not enough to maintain good growth and survival of *L. vannamei* in the grow-out phase without addition of artificial feed. When artificial feed was added, even in low feeding rates, the survival increased and the shrimp partially recovered their growth capacities. These results may be related to the high nutritional value of the aggregates present in the biofloc and microorganisms present in the biofilm. The biofilm contributed to the achievement of higher yields without the need to increase the feeding rates, making the system more sustainable in both economic and environmental terms, reducing feeding costs and enhancing the nutrient recycling.

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CONCLUSÕES GERAIS:

De acordo com os resultados obtidos nos diferentes experimentos da presente tese é possível afirmar que a produtividade natural provida pelo sistema de bioflocos pode contribuir com uma maior sustentabilidade do sistema utilizando diferentes técnicas de manejo alimentar para melhorar o crescimento dos animais. Um importante ponto evidenciado nos estudos é de que as quantidades de ração fornecidas podem ser reduzidas nesse sistema, obtendo as mesmas produtividades, evidenciando o papel dos bioflocos na nutrição dos camarões. Assim, de acordo com a metodologia de alimentação proposta, indica-se que no sistema BFT não há necessidade de se aumentar as taxas de arraçoamento com taxas fixas de conversão alimentar de 1,6 para 2,0, visto que o crescimento dos camarões é o mesmo, reduzindo em aproximadamente 25% a quantidade de ração a ser fornecida. Além disso, é possível a manutenção de camarões durante longos períodos (60 dias) com taxas de arraçoamento baixas, sem perdas na sobrevivência, indicando que possivelmente o sistema de bioflocos dá condições aos animais para sobreviver em elevadas densidades em condições de pouco alimento artificial.

Adicionalmente, períodos longos de restrição alimentar (21 dias), seguidos por um período de 29 dias de realimentação evidenciou que camarões cultivados com pequenas quantidades de alimento podem compensar parcialmente o peso perdido durante o período de jejum. Mais uma vez, no entanto, foi evidenciado que exceto para os animais que não receberam nenhuma quantidade de alimento durante 21 dias, a manutenção dos camarões sob baixas taxas de arraçoamento é possível sem perdas na sobrevivência. Igualmente, utilizando-se da técnica de restrição alimentar seguida de realimentação, foi possível obter uma economia de aproximadamente 25% de ração. Seguindo a mesma linha de estudo, mas utilizando períodos mais curtos de restrição alimentar (2 e 4 dias na semana) seguidos de períodos de realimentação em regimes cíclicos de alimentação, foi possível observar que camarões que foram alimentados por 5 dias na semana com as mesmas quantidades do que os animais controle apresentaram hiperfagia, e isso permitiu que os mesmos atingissem crescimento compensatório completo comparando-se os pesos finais entre esses dois esquemas de alimentação. Além disso, a redução de dias de alimentação (de 7 para 5 dias na semana) juntamente com as quantidades de ração fornecidas (quantidade a ser fornecida apenas durante os 5 dias) não afetou a produtividade final do cultivo, evidenciando o papel dos bioflocos como fonte alternativa de alimento quando os camarões são cultivados com menores quantidades de alimento artificial.

Finalmente, aliando-se a tecnologia de bioflocos com a utilização de substratos artificiais para a fixação de biofilme torna possível reduzir ainda mais as quantidades de alimento. A redução nas quantidades de alimento artificial quando se utiliza biofilme pode chegar próximo de até 35% do total de ração oferecido, representando uma grande economia nos custos de produção.

Dessa forma, considerando-se a genética de crescimento de pós-larvas de camarão e a qualidade do alimento artificial disponíveis no mercado nacional, juntamente com a manutenção da qualidade de água em condições dentro dos limites indicados para o camarão *Litopenaeus vannamei* no sistema de bioflocos, pode-se indicar que a taxa de conversão alimentar mínima estimada para um cultivo com densidades entre 300 e 400 camarões/m² é de 1,1, e o valor máximo sem utilização de biofilme é de 1,8. Outro ponto importante do ponto de vista de manejo de cultivo, é que os animais podem ser cultivados com baixas taxas de alimento durante períodos de 21 a 60 dias, o que pode ser uma técnica utilizada em casos de deterioração da qualidade de água que leve à redução das taxas de arraçoamento (por exemplo, amônia e nitrito elevados, oxigênio dissolvido baixo) sem perdas significativas na sobrevivência devido às baixas taxas de arraçoamento. Além disso, também é necessário evidenciar que o protocolo de alimentação pode ser aplicado por produtores, desde que os mesmos já conheçam os padrões de crescimento da espécie dentro de seu sistema de cultivo.

Anexo 1 – Tabela com valores recomendados para a produção de *Litopenaeus vannamei* em sistema de bioflocos sob condições controladas rotineiramente utilizadas na produção na Estação Marinha de Aquacultura da Universidade Federal do Rio Grande (EMA-FURG).

Densidades de estocagem	Entre 300 e 400 camarões/m³
Taxa de conversão alimentar mínima	1,1
Taxa de conversão alimentar máxima	1,8
Taxa de crescimento semanal média	Entre 1 e 1,1 g/semana
Mortalidades esperadas	0,5%/semana
Utilização de biofilme (custo elevado, porém alternativa sustentável devido a seus benefícios nutricionais e para a qualidade de água)	
Redução de custos com ração e mão de obra	