UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CENTRO DE AQUICULTURA DA UNESP

Berçário de tilápia em sistema aquapônico utilizando a tecnologia de bioflocos

Sara Mello Pinho

Jaboticabal, São Paulo
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RESUMO

Empregar a tecnologia de bioflocos na produção aquapônica, integrando o cultivo de alface com o berçário de tilápia, parece ser uma alternativa para solucionar a problemática relacionada ao abastecimento de juvenis de tilápia à engorda, por meio de sistemas de produção responsáveis, menos impactantes em termos ambientais e economicamente viáveis. Além disso, o consumo dos agregados microbianos dos bioflocos pelos peixes poderá possibilitar a produção de juvenis diferenciados e com condições nutricionais desejáveis. Sabendo disso, foi proposto avaliar a produção aquapônica de juvenis de tilápia (~ 1 até 30 g) e alface lisa utilizando a tecnologia de bioflocos. Foram avaliados dois tipos de tecnologias aquícolas: recirculação de água clara (CW, ou controle) e bioflocos (BFT), com três repetições cada. O experimento foi executado durante 46 dias, divididos em dois ciclos de produção de alface (23 dias cada) e um ciclo de produção de juvenis de peixe. Buscou-se relacionar os dados de desempenho produtivo com análises zootécnicas e fitotécnicas; das características visuais das plantas; das concentrações dos nutrientes que entram e saem dos sistemas; bem como com avaliações morfológicas e morfométricas das fibras musculares e da composição centesimal dos peixes cultivados. Análise de viabilidade econômica também foi realizada, avaliando quatro cenários: dois levando em conta a porcentagem de alfaces comercializáveis encontradas no experimento pelo índice de qualidade da planta (BFT: 37% e CW: 98% do total) e outros dois, considerando uma produção hipotética em que todas as alfaces produzidas apresentam qualidade para serem vendidas. Os resultados mostraram melhor desempenho produtivo dos peixes cultivados em BFT. Os dados da trajetória de crescimento em peso, crescimento das fibras musculares e composição centesimal dos peixes mostraram resultados similares entre CW e BFT. Enquanto que para as plantas, o tratamento CW no ciclo 2 foi o que apresentou melhores resultados fitotécnicos e visuais. A análise econômica indicou que é viável a produção aquapônica utilizando CW ou BFT, desde que neste último o mínimo de 63,5% das plantas produzidas seja visualmente aptas para comercialização. De maneira geral, os resultados sugerem que a integração do BFT com a aquaponia poderá ser viável somente a partir do desenvolvimento de mecanismos que possibilitem diminuir o acúmulo de sólidos nas raízes e melhorar a absorção dos nutrientes pelas plantas.

Palavras-chave: tilapicultura, hidroponia, integração, nutrição, viabilidade econômica.

ABSTRACT

The use of biofloc technology in aquaponic production, integrating lettuce production with tilapia nursery, seems to be an alternative to solve the problems related to the supply of juveniles for the grow-out stage, by means of responsible, less environmentally impacting and economically feasible production systems. Besides, consumption of the microbial aggregates present in the biofloc may enable the production of differential juveniles, with desired nutritional conditions. In this sense, this study aimed at evaluating the aquaponic production of tilapia juveniles (~ 1 to 30 g) and lettuce, using biofloc technology. For this purpose, two aquaculture technologies were studied: clear water recirculation (CW, or control) and biofloc (BFT), with three replicates each. The experiment was carried out for 46 days, with two cycles of lettuce production (23 days each) and one cycle of juvenile production. We sought to relate the productive performance data with zootechnical and phytotechnical analyzes; visual characteristics of plants; concentration of nutrients entering and leaving the system; as well as morphological and morphometric analyzes of muscle fibers and centesimal composition of fish. An economic feasibility analysis was also performed, considering four scenarios: two considering the percentage of commercially available lettuce found in the experiment, by the plant quality index (BFT: 37% and CW: 98% of the total) and two others, considering a hypothetical production in which all produced lettuces had good quality to be commercialized. Results showed better productive performance of fish reared in BFT. The data of the growth trajectory in weight, muscle fibers and centesimal composition of fish were similar between CW and BFT. Conversely, for the plants, the treatment CW in the second cycle presented the best phytotechnical and visual results. The economic analysis indicated that the aquaponic production is feasible using both CW and BFT, provided that in BFT a minimum of 63.5% of produced plants are visually suitable for marketing. Generally, the results suggest that the integration BFT with aquaponics may be feasible only through the development of mechanisms that enable reduced accumulation of solids in the roots, thus improving the absorption of nutrients by the plants.

Keywords: tilapia farming, hydroponics, integration nutrition, economic feasibility.

1. INTRODUÇÃO GERAL

1.1. Tilapicultura no Brasil

A piscicultura continental representa mais de dois terços da produção aquícola brasileira. Estima-se que em 2016 foram produzidas, aproximadamente, 507 mil toneladas de peixes de água doce no Brasil, movimentando cerca de R$ 3,26 bilhões (IBGE, 2017). Entre as principais espécies cultivadas, a tilápia destaca-se devido, principalmente, às boas taxas de crescimento, rusticidade, domínio da reprodução e mercado consolidado (Watanabe et al., 2012). Em 2016 a tilapicultura contribuiu com aproximadamente 239 mil toneladas de peixes despescados (IBGE, 2017), grande parte desse volume provém de tanques-rede alocados em grandes reservatórios da União (Leonardo et al., 2009). No entanto, diversos desafios são apontados para garantir o crescimento da atividade, dentre eles, a obtenção de juvenis de boa qualidade (Ayroza et al., 2011). Neste sentido, o berçário, ou a produção de juvenis de 30 g (comercialmente chamado de "alevinão"), é uma etapa extremamente importante, pois permite oferecer à engorda peixes com tamanho adequado para povoamento nas estruturas produtivas. Além disso, a segmentação da produção, principalmente a separação do berçário da engorda, possibilita maior controle de enfermidades, eficiência produtiva e gestão do escalamento do cultivo (Little et al., 2003). Embora seja dominada por cultivos monofásicos, a utilização de berçários ou cultivos multifásicos já é uma realidade na piscicultura brasileira. Em 2016, houve incremento de 14% na produção nacional de juvenis em relação ao ano anterior (IBGE, 2017), cultivados principalmente em viveiros escavados e hapas.

Outro desafio enfrentado pela tilapicultura é o fato que os cultivos tradicionais, quando manejados de forma incorreta, podem provocar problemas ambientais e econômicos. Por exemplo, pode-se citar a descarga de efluentes ricos em nutrientes dos cultivos em viveiros escavados (Abimorad et al., 2009); ou ainda a disseminação de patógenos e acúmulo de material orgânico em regiões utilizadas pelos cultivos em tanques-rede (Salvador et al., 2003). Por causa disso, é crescente o interesse na aplicação de manejos ou sistemas que visem atingir elevadas produtividades com mínimo impacto ambiental (Martins et al., 2010; Rijn, 2013). Somado a isso, o panorama atual de secas, o aumento no custo das terras e a necessidade de cultivos urbanos e próximos ao mercado consumidor, fazem com que produtores e pesquisadores busquem alternativas para a

produção de alimentos, tais como os sistemas de bioflocos e a aquaponia (Emerenciano et al., 2015).

1.2. Bioflocos

A tecnologia de bioflocos ou "BFT" (do inglês "*Biofloc Technology")* foi desenvolvida na década de 1970 pelo IFREMER-COP (Instituto Francês de Investigação para a Exploração do Mar, Centro Oceanográfico do Pacífico), tendo como principal característica a troca mínima ou nula de água e o crescimento de comunidades microbianas específicas (Emerenciano et al., 2013). Inicialmente aplicado na carcinicultura, hoje o BFT também está presente na tilapicultura, havendo aumento expressivo das pesquisas publicadas sobre tilápia em BFT a partir de 2006 (Figura 1).

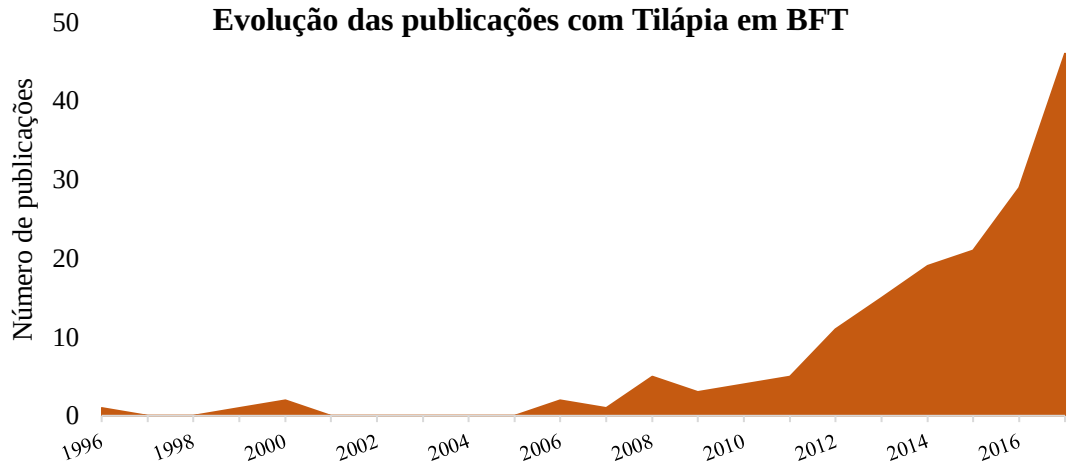


Figura 1. Resultado da busca na plataforma Scopus, com a palavra-chave "*tilapia biofloc*", mostrando a evolução dos artigos publicados em periódicos científicos.

Nas unidades produtivas, para fomentar a microbiota desejada, estimula-se o crescimento de bactérias heterotróficas via manipulação da relação carbono:nitrogênio (C:N) entre 12 a 20:1, forte aeração e movimentação constante da água (Crab et al., 2012; Avnimelech, 2015). Esta microbiota, por sua vez, confere maior estabilidade ao sistema, incorporando a amônia excretada pelos organismos cultivados em biomassa bacteriana, fomentando a "alça microbiana" (Thompson et al., 2002; Ray et al., 2010; Crab et al., 2012). Além das bactérias heterotróficas, bactérias quimioautotróficas e organismos planctônicos, principalmente microalgas, protozoários e rotíferos, são também

constantemente relatadas nos cultivos de tilápia em BFT e auxiliam na ciclagem dos nutrientes (Martínez-Córdova et al., 2014). No entanto, o perfil da microbiota dos flocos pode apresentar grande variação, devido a fatores como a espécie produzida e seu hábito alimentar, fonte de carbono utilizada, condições ambientais de cultivo e interação ou competição com outros microrganismos (Martínez-Córdova et al., 2014; Luo et al., 2017).

Mesmo com as variações do perfil das comunidades, de maneira geral, os agregados microbianos do BFT são ricos em nutrientes, como proteínas, aminoácidos essenciais, ácidos graxos e vitaminas, e complementam a dieta alimentar dos peixes cultivados (Ju et al., 2008; Wei et al., 2016; Bossier e Ekasary, 2017). Em razão da presença desses organismos, a tecnologia de bioflocos proporciona vários benefícios a produção aquícola, tais como i) disponibilidade contínua de alimento natural e consequente melhoria da conversão alimentar; ii) aumento do controle e biossegurança dos cultivos; iii) menor dependência de grandes volumes de água para produção; iv) manutenção da qualidade da água, principalmente em relação aos compostos nitrogenados; v) melhora na saúde do animal, pela competição biológica com patógenos e a consequente inibição da atuação destes (Emerenciano et al., 2017). O consumo da rica microbiota disponível no BFT pode proporcionar também juvenis diferenciados (Bossier e Ekasary, 2017). A disponibilidade constante de alimento natural, além de complementar as demandas nutricionais (Martínez-Córdova et al., 2014), poderá alterar o desenvolvimento muscular dos peixes cultivados. Isto porque, o manejo alimentar pode ocasionar alterações nos padrões de hipertrofia (aumento no volume) e hiperplasia (formação de novas fibras) das fibras musculares, especialmente nas fases iniciais de cultivo (Johnston, 2006; Almeida et al., 2008; Kojima et al., 2015), podendo levar a efeitos nas fases posteriores de criação, ou seja, proporcionar peixes diferenciados devido ao maior potencial de crescimento muscular futuro (Valente et al., 2013). No entanto, ainda não está elucidado o efeito da produtividade primária dos sistemas de bioflocos na ativação dos processos de crescimento das fibras musculares.

O cultivo de tilápias em bioflocos tem inúmeras vantagens e estas refletem na melhora do desempenho zootécnico do animal. Azim e Little (2008) relatam produção de tilápia 45% superior em BFT em relação aos tanques de água clara e, por sua vez, Luo et al. (2014) obtiveram redução na conversão alimentar de juvenis de tilápia de 1,47 para 1,20, comparando sistema de bioflocos ao de água clara. Na Tabela 1 estão apresentados alguns resultados zootécnicos registrados na literatura para juvenis de tilápia-do-nilo

(*Oreochromis niloticus*) em BFT. Nota-se que os valores de sobrevivência, conversão alimentar (CA) e taxa de crescimento específico (TCE) para a fase de berçário são, na maioria dos casos, próximos a 80%, 1,0 e 3,7% dia^{-1}, respectivamente (Zhang et al., 2016; Brol et al., 2017; Martins et al., 2017; Miranda-Baeza et al., 2017). Observa-se também grande variação entre os resultados, podendo estar relacionada a fatores como o manejo alimentar, densidade de estocagem e peso inicial, mas também as condições ambientais ou as diferenças no aporte de alimento complementar (floco microbiano).

Tabela 1. Revisão dos resultados zootécnicos mais recentes de juvenis de tilápia-do-nilo cultivados em sistema de bioflocos.

Referência	Peso inicial (g)	Densidade (peixes m^{-3})	Duração (dias)	Manejo alimentar*	Resultados zootécnicos		
					SOB (%)	CA	TCE (% dia^{-1})
Luo et al. (2014)	24,2	330	87	2% PV 3x dia 44% PB	100	1,20	2,13
Long et al. (2015)	50,6	60	56	3% PV 2x dia 46% PB	100	0,83	2,04
Pérez-Fuentes et al. (2016)	38,4	75	120	3% PV 2x dia 45% PB	85 - 95	1,02 - 1,14	1,49 - 1,65
Zhang et al. (2016)	13,6	240	120	2% PV 3x dia 30% PB	81	0,85	2,25
Brol et al. (2017)	3,1	400 e 800	42	Saciedade 3x dia 45% PB	73 - 87	1,21 - 1,29	4,46 - 4,51
Mansour e Esteban (2017)	48	42	70	3% PV 2x dia 20 a 30% PB	98 - 100	1,72 - 1,80	1,02 - 1,05
Martins et al. (2017)	3,7	25	60	15 a 4% PV 4x dia 45 a 40% PB	80 - 81	1,10 - 1,18	5,31 - 5,45
Miranda-Baeza et al. (2017)	14,5	20	35	Saciedade Não informado 55% PB	98 - 72	1,35 - 1,85	2,77 - 3,17
Pinho et al. (2017a)	70,0	80	21	5% PV 3x dia 22% PB	95	1,87	1,88

* Taxa de alimentação (% peso vivo), frequência alimentar (vezes por dia) e concentração de proteína bruta (% PB) nas dietas.

Mesmo que o BFT esteja sendo amplamente empregada para intensificação aquícola (Bossier e Ekasary, 2017), ele apresenta entraves. A necessidade constante de energia elétrica, monitoramento frequente dos parâmetros físico-químicos da água e mão-de-obra especializada são alguns exemplos. Além disso, a baixa renovação de água e as altas densidades empregadas (Crab et al., 2012; Avnimelech, 2015) podem gerar grande concentração de nutrientes nas águas do cultivo (Quintã et al., 2015), prejudicando sua qualidade e a eficiência da produção. Neste sentido, a produção vegetal em um sistema aquapônico integrado ao cultivo de juvenis de tilápia em BFT pode ser uma alternativa para minimizar estes problemas (Buhmann et al., 2015; Pinho et al., 2017a).

1.3. Aquaponia

A técnica consiste na integração do sistema de recirculação aquícola (cultivo intensivo de organismos aquáticos) com a hidroponia (cultivo de plantas terrestres em solução aquosa) (Rakocy, 2012). Esta técnica é possível via um sinergismo com os microrganismos presentes no sistema, os quais convertem os efluentes aquícolas, principalmente de amônia para nitrato, em nutrientes para as plantas (Tyson et al., 2011; Ru et al., 2017). Com isso os nutrientes residuais do cultivo de peixes são naturalmente transformados em produtos absorvíveis pelas plantas, gerando um subproduto comercial de valor agregado, ao mesmo tempo que mantem a qualidade da água (Endut et al., 2010; Moya et al., 2014) (Figura 2).

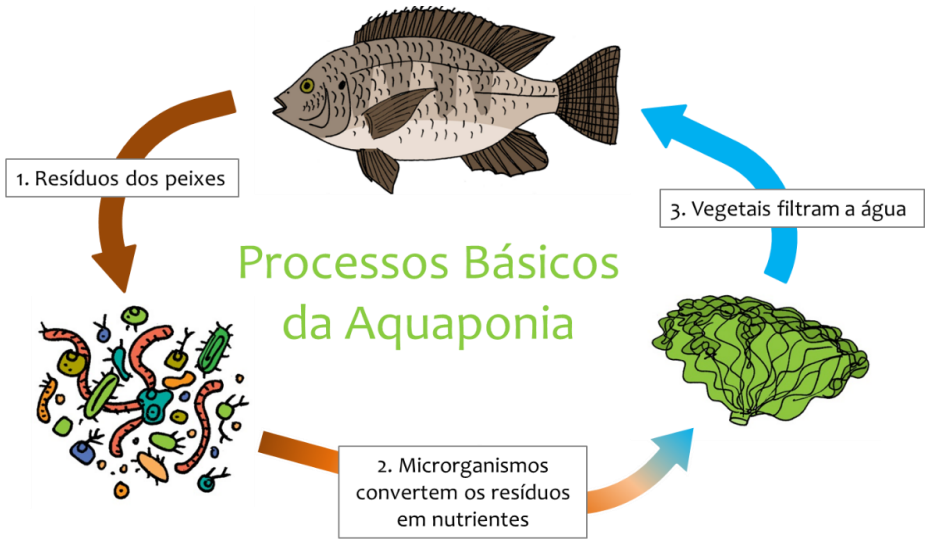


Figura 2. Ilustração dos processos básicos que ocorrem em sistemas aquapônicos.

Desde 1970, pesquisadores da Universidade das Ilhas Virgens Americanas, em especial o Dr. James Rakocy, já testavam plantas como filtros naturais na aquicultura, desenvolvendo os primeiros conceitos e dimensionamentos básicos da aquaponia moderna (Goddek et al., 2015; Emerenciano et al., 2015). Atualmente, em virtude das várias vantagens (Tabela 2), a aquaponia é reconhecida como uma prática mais sustentável para produção de peixes e plantas (Goddek et al., 2015; Forchino et al., 2017). É praticada tanto em escala "hobbie", em ambientes pequenos e familiares, como em grandes estruturas com fins comerciais. Em escala comercial o objetivo na maioria das vezes é conquistar o público que reconheça o caráter ecológico dos produtos e que esteja disposto a pagar mais pelo valor agregado (Edwards, 2015).

Tabela 2. Principais vantagens e desvantagens do sistema de aquaponia (adaptado de Emerenciano et al., 2015).

Vantagens	**Desvantagens**
Uso racional da água	Tecnologia pouco difundida no Brasil
Alta produtividade de peixes e vegetais	Dependência contínua de energia elétrica
Produtos livres de agrotóxicos e antibióticos (valor agregado)	Necessidade de mão-de-obra especializada (peixes e plantas)
Aproveitamento praticamente integral da ração	Custos de implantação relativamente elevados
Possibilidade de produção em zonas áridas e centros urbanos	Necessidade de condições ambientais iguais para peixes e plantas
Diversidade na produção (menores riscos com a flutuação do mercado)	Necessidade de controle e monitoramento constante
Baixo risco de introdução de espécies exógenas ao cultivo	
Instrumento de educação em escolas e inclusão social	

Apesar de todas essas vantagens que os sistemas aquapônicos podem apresentar, o investimento para a implantação é alto em comparação às estruturas tradicionais aquícolas, principalmente devido a adição das bancadas hidropônicas e dos sistemas de filtragem (Tokunaga et al., 2015; Bosma et al., 2017). A bancada ou estrutura hidropônica representa o local onde as plantas são distribuídas, para que tenham contato com a água e consequentemente com os nutrientes (Rakocy, 2012). Existem três principais modelos de aquaponia, caracterizados pelo tipo de bancada hidropônica, conhecidas como: substrato semi-seco, NFT (do inglês "*Nutrient Film Technology*") e bandejas flutuantes (Emerenciano et al., 2015). Este último, também conhecido como sistema *floating*, é o mais utilizado em escalas comerciais pela facilidade no plantio e colheita, por possuir melhor custo-benefício de implantação e apresentar menores variações dos parâmetros de qualidade de água (Lennard e Leonard, 2006). Os filtros, mecânicos e biológico, por sua vez, são os mesmos característicos da produção aquícola intensiva em recirculação (Lekang, 2007) e devem ser dimensionados corretamente para que haja melhor aproveitamento dos nutrientes pelas plantas.

Um ponto importante para o sucesso da aquaponia, muitas vezes visto como um entrave, é a busca pelo equilíbrio entre as condições abióticas do sistema, de forma a maximizar o desempenho dos organizamos cultivados. As condições ambientais, tais como temperatura, pH e concentração dos minerais na água, devem estar dentro ou o mais próximo possível das faixas ótimas para o desenvolvimento das plantas, peixes e microrganismos (Goddek et al., 2015). Para isso, o entendimento da relação entre eles e a escolha correta das espécies a serem cultivadas são fundamentais (Knaus e Palm, 2017). Entre os organismos aquáticos, a maior parte dos trabalhos científicos e empresas especializadas sugerem o uso das tilápias (Dediu et al., 2012; Love et al., 2015). Entretanto, outras espécies também aparecem com potencial para serem utilizadas na aquaponia, como o bagre africano (Endut et al., 2010; Palm et al., 2014), as carpas (Haque et al., 2015; Shete et al., 2017), truta arco-íris (Forchino et al., 2017), robalo europeu (Nozzi et al., 2016), pacu (Pinho et al., 2017b) ou até mesmo os camarões branco do pacífico (Pinheiro et al., 2017) e gigante da Malásia (Sace e Fitzsimmons, 2013). Em relação as plantas, mais de 150 espécies de vegetais demostraram bons resultados ao serem cultivados na aquaponia (FAO, 2014). Al-Hafedh et al. (2008) e Love et al. (2015) citam que flores, frutos e ervas aromáticas (temperos) são cultivados no sistema aquapônico com sucesso. No entanto, hortaliças folhosas como a alface (*Lactuca sativa*)

são as mais indicadas pois possuem boa aceitação de mercado, rápido crescimento, baixo requerimento nutricional e excelente adaptação ao sistema (Diver, 2006; Rakocy, 2012; Sala e Costa, 2012). Normalmente, todos os nutrientes exigidos pelas alfaces são encontrados em níveis adequados na água de cultivo, podendo alcançar o mesmo desempenho que as cultivadas na hidroponia ou, quando os cultivos aquapônicos são suplementadas com minerais, apresentar índices produtivos superiores aos da alface hidropônica (Delaide et al., 2016).

1.4. Bioflocos x Aquaponia

A aquaponia poderia se tornar mais eficiente ao beneficiar-se do "pool microbiano" através do aproveitamento dos efluentes dos sistemas de bioflocos (Emerenciano et al., 2013). Entretanto, poucos são os relados da integração da aquaponia com o BFT. Em estudo recente, Pinho et al. (2017a) relatam que três variedades de alface (lisa, crespa e roxa) apresentaram melhores desempenhos ao serem cultivadas utilizando o efluente do cultivo de tilápia em BFT, em comparação ao sistema de recirculação em água clara. No entanto, embora tenha um forte caráter inovador e seja o primeiro relato sobre a integração entre ambas tecnologias para tilápia e alface, o curto período do estudo provavelmente não refletiu uma situação real de cultivo, quando o floco já esteja maduro ou estabelecido. Portanto, faz-se necessário estudos mais aprofundados sobre a utilização da tecnologia de bioflocos na produção aquapônica, visto que esta integração parece ser uma alternativa interessante. Isto porque, considera-se que a integração entre as duas tecnologias permite produzir uma grande biomassa de peixes e plantas em pequenos espaços, com menos impacto ambiental que os monocultivos tradicionais (Emerenciano et al., 2015). Além disso, o emprego do BFT na produção de alface aquapônica pode não só potencializar a produção dos peixes (Emerenciano et al., 2013), mas também fazer com que a comunidade microbiana presente nesta tecnologia melhore a disponibilidade ou auxilie na absorção dos nutrientes pelas plantas (Pinho et al., 2017a). Como resultado, além do incremento de renda em função do valor ecológico agregado, a integração também poderá contribuir para aumentar a oferta de juvenis diferenciados no mercado (Bossier e Ekasary, 2017), impulsionando o elo seguinte da cadeira produtiva, ou seja, a engorda, (Figura 3).

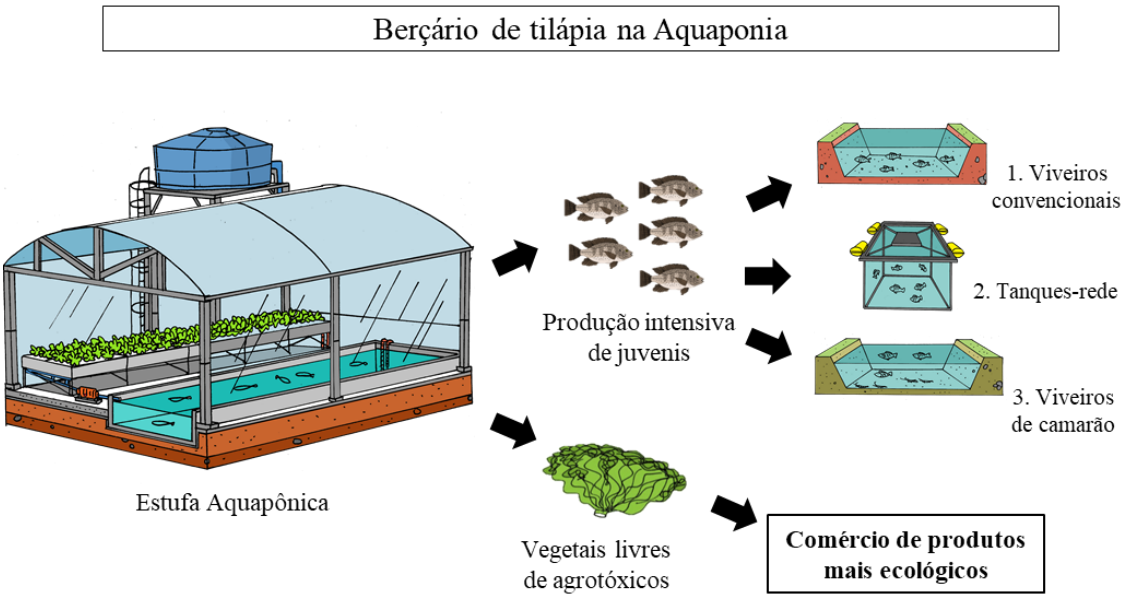


Figura 3. Representação do emprego da tecnologia de bioflocos na produção aquapônica de juvenis de tilápia, visando o povoamento das estruturas de engorda além do incremento da renda com a comercialização de vegetais sem agrotóxicos.

Empregar a tecnologia de bioflocos na produção aquapônica, integrando o cultivo de alface com o berçário de tilápia (~ 1 g até 30 g), parece ser uma ótima alternativa para solucionar a problemática relacionada ao abastecimento de juvenis de tilápia à engorda, por meio de sistemas de produção responsáveis, menos ambientalmente impactantes e economicamente viáveis. É oportuno salientar que o desenvolvimento de novas tecnologias é importante para consolidar a produção intensiva e ambientalmente responsável de tilápias. Além disso, integrar a aquicultura com a produção de vegetais é uma alternativa economicamente interessante, visto que as plantas podem representar até 80% dos lucros da produção (Emerenciano et al., 2015; Tokunaga et al., 2015). Pelo exposto, torna-se fundamental o correto entendimento entre o desempenho produtivo dos peixes e das plantas, a dinâmica dos nutrientes no sistema, a qualidade dos juvenis cultivados e a viabilidade econômica da integração, de forma a avaliar se a integração do sistema BFT e aquapônico é viável.

2. OBJETIVOS

2.1. Objetivo Geral

Avaliar a possibilidade de integrar a produção de alface em sistema aquapônico com a produção de juvenis de tilápia-do-nilo (*Oreochromis niloticus*) utilizando a tecnologia de bioflocos.

2.2. Objetivos específicos

- Avaliar o desempenho zootécnico, na fase de berçário, de juvenis de tilápia-do-nilo criados na aquaponia em sistema de bioflocos e recirculação de água clara;
- Caracterizar o perfil da comunidade planctônica da água no sistema de bioflocos e recirculação de água clara;
- Avaliar o desempenho fitotécnico e as características visuais das plantas aquapônicas, cultivadas com efluentes do sistema de bioflocos e recirculação de água clara;
- Avaliar as entradas e saídas dos nutrientes na aquaponia em sistema de bioflocos e recirculação de água clara;
- Analisar a morfologia e morfometria das fibras musculares dos peixes cultivados na aquaponia em sistema de bioflocos e recirculação de água clara;
- Avaliar a composição centesimal dos peixes cultivados na aquaponia em sistema de bioflocos e recirculação de água clara;
- Analisar a viabilidade econômica do sistema integrado bioflocos e aquaponia.

3. HIPÓTESE

A integração do sistema de aquaponia com a tecnologia de bioflocos proporcionará melhor eficiência produtiva dos juvenis de tilápia-do-nilo e alface, de maneira rentável e sem afetar os benefícios nutricionais que o consumo dos flocos microbianos proporciona aos juvenis.



Artigo 1 - Dissertação Sara Pinho

4. ARTIGO 1

PRODUCTION OF NILE TILAPIA JUVENILES AND LETTUCE IN AN AQUAPONIC SYSTEM USING BIOFLOC TECHNOLOGY

O artigo foi formatado segundo as normas da revista *Ecological Engineering* QUALIS CAPES A2 na área de Zootecnia e Recursos Pesqueiros, com fator de impacto igual a 2,91.

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Production of Nile tilapia juveniles and lettuce in an aquaponic system using biofloc technology

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Abstract

The possibility of producing lettuce and tilapia juveniles in an aquaponic system using effluent from a biofloc system could help to increase the efficiency of both systems. The aim of the present study was to evaluate the aquaponic production of lettuce (*Lactuca sativa*, var. butter) and nursery stage (~ 1 to 30 g) of Nile tilapia juveniles (*Oreochromis niloticus*) using biofloc technology (BFT) and clear-water recirculation (CW) as a control treatment. The experiment was carried out for 46 days and was divided into two cycles of lettuce production (23 days each) and one cycle of juvenile production. The physical-chemical variables of the water, zootechnical performance of the fish, phytotechnical performance and visual characteristics of the plants, nutrient dynamics, and characterization of the planktonic community were evaluated in both technologies. The results showed better fish productive performance in BFT. For plants, cycle 2 in CW showed the best phytotechnical and visual results (98% of the plants received grades A and B for plant quality index), whereas in the BFT only 37% achieved these grades in the same cycle. The results suggest that the integrated production of *Oreochromis niloticus* juveniles and lettuce using BFT might be feasible only via the development of mechanisms to decrease the accumulation of solids in the lettuce roots, improving the absorption of nutrients by plants.

Keywords: integration; nursery; *Lactuca sativa;* nutrient

1. Introduction

Biofloc technology (BFT) has been used for the intensification of aquaculture production (Emerenciano et al., 2017). Minimal water exchange and manipulation of the carbon and nitrogen ratio (C:N) of water by the addition of external carbon sources ensures that microbial communities beneficial to fish and shrimp rearing are favored within the culture tanks (Martínez-Córdova et al., 2016). These microorganisms, mainly microalgae, rotifers, protozoa, and nitrifying and heterotrophic bacteria (Martínez-Córdova et al., 2014; Ray et al., 2010), are important for the maintenance of water quality. Besides their key roles in the recycling of organic matter and residual nitrogen compounds, these microbiota are *in-situ* protein synthesizers that are utilized as supplementary food and for improving zootechnical performance (Avnimelech, 2015; Crab et al., 2012). Initially developed as an alternative to solve several diseases outbreaks in shrimp farming due to its higher biosecurity and beneficial bacteria microbiota capable to control the development of pathogens, BFT has also been strongly considered for tilapia rearing (Emerenciano et al., 2013a; Brol et al 2017). Azim and Little (2008) reported 45% higher Nile tilapia production in BFT than in clear-water recirculation (CW) systems. Luo et al. (2014) observed that feed conversion of tilapia juveniles was lower in clear-water (1.47) than in the BFT (1.20). Therefore, the use of BFT to produce tilapia juveniles (1 to 30 g) can be a strategy to segment and dynamize tilapia culture, considering that the species utilizes efficiently the primary productivity during the nursery stage (Alves et al., 2017).

BFT does not only provide zootechnical advantages for aquaculture organisms but is also recognized as an environment friendly system as it does not depend on high water renewal or large areas of land (Crab et al., 2012). However, these factors, together with the high stocking density practiced in intensive aquaculture systems, can generate high concentrations of nutrients in the water (Quintã et al., 2015). Therefore, one option for the use of the nutrients generated by BFT is integration with the aquaponic system (Buhmann et al., 2015; Pinho et al., 2017). Aquaponics is the production of fish/shrimp and plants in a single system by the integration of a recirculation aquaculture system with hydroponics. By biological filtration, microorganisms (mainly nitrifying bacteria) convert the residual effluents of the fish culture into nutrients absorbable by plants, providing the maintenance of water quality (Endut et al., 2010; Moya et al., 2014; Schmautz et al., 2016).

Plant production in aquaponic system is directly related to the supply of fish feed, its nutritional characteristics, rate of mineralization by microorganisms, and the requirements and nutrient absorption capacity of different plant species (Goddek et al., 2015). The microbial community present in the BFT could improve the availability or help the absorption of nutrients present in the aquaponics system (Pinho et al., 2017). Pinho et al. (2017) showed that three varieties of lettuce grown in an integrated system with tilapia displayed better performances when cultivated in BFT than in clear-water, especially the butter variety.

The possibility of producing lettuce and tilapia juveniles in aquaponics system using BFT effluent can increase the productivity and diversification of these systems. In addition, the better use of feed, and consequently, the lowest concentration of nutrients in the effluent can increase the sustainable characteristics of production. Therefore, the aim of the present study was to evaluate the use of biofloc technology (BFT) and clear-water recirculation (CW) for aquaponic production of butter lettuce and Nile tilapia juveniles.

2. Material and methods

The experiment was carried out in the Aquaculture Laboratory (LAQ) at the Santa Catarina State University (UDESC) in Laguna, Santa Catarina, Brazil, under the authorization of the Committee on Ethics in Animal Use (CEUA – Protocol No. 17615/16). The experiment was performed in a greenhouse (18 m^2, 3 m height), covered with a 1.5 mm plastic liner, and 50% of its luminosity reduced using sunshade net.

2.1. Experimental design

Two types of aquaculture technologies were evaluated: clear-water recirculation (CW) and biofloc (BFT) for the aquaponic production of Nile tilapia juveniles (*Oreochromis niloticus* at the nursery stage, ~ 1 to 30 g) and butter lettuce (*Lactuca sativa).* The experiment was conducted for 46 days, comprising two cycles of lettuce production (23 days each) and one cycle of juvenile fish production. Each technology characterized one treatment, replicated in six independent recirculation systems: three using CW and three using BFT.

Each system consisted of one circular plastic tank (500 L) used for fish culture and three rectangular plastic tanks (0.5 m^2 and 30 cm high) used as experimental units for the plants (hydroponic system) (Figure 1). An expanded polystyrene block (18 kg m^{-3}

density, 30 mm) with area identical to each tank was placed on the surface of each tank for plant production. The design of the CW system followed the features of a conventional recirculation system, comprising by mechanical filters (conical sedimentation or clarifier (15 L) and bag (Perlon®)), and biological filter (with PET caps as substrate for nitrifying bacteria, aerated with a 20 cm long Aerotube® microperforated diffuser) installed in the same tank as the sump (70 L circular plastic tank). In the BFT, two conical sedimentation filters (or clarifiers) were placed after the fish tank aiming to reduce the accumulation of particulate matter (biofloc) in the plant tanks. In addition, an airlift system was used at the bottom of each clarifier to attempt the return of the settled biofloc to the fish tank. Powder molasses was added daily as a complementary carbon source to maintain the desired C:N ratio (20:1) in the BFT system (methodology adapted from Emerenciano et al., 2017).

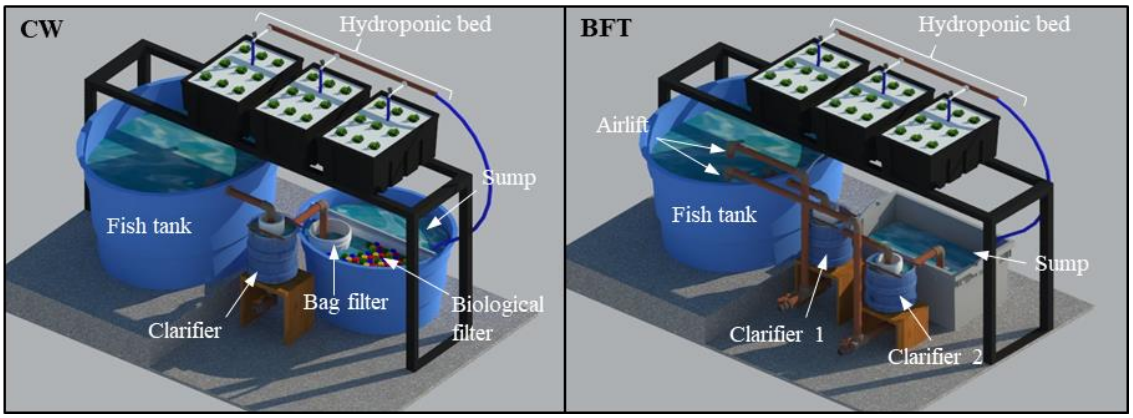


Figure 1. Schematic design of the clear-water recirculation (CW) and biofloc technology (BFT) systems used in the aquaponic experimental culture of juvenile tilapia and butter lettuce.

In both technologies, water from the fish tank was gravity fed through the filters until it reached the sump, where a submersible pump (45 W, 2700 L h^{-1}) pumped the water to the hydroponic system (~ 8 L min^{-1} water flow) and returned it by gravity to the fish tank (Figure 1). The clarifiers were constantly used in both systems. To maintain a constant water temperature close to 27 °C, two thermostats (500 W, Roxin HT-1900, Rongxingda Aquarium Equipment Factory, Guangdong, China) were used in each system. No water was exchanged during the experiment, only dechlorinated freshwater was added to compensate for evaporation loss.

Aeration was provided by an air blower (2 HP) and distributed in each system by a circular micro-perforated diffuser (25 cm Ø, Aerotube®) in the fish tanks, one air-stone (20 mm Ø, 30 mm height) placed in the center of each plant tank, with a micro-perforated diffuser (26 cm length) in the CW biological filter, and two hoses (10 mm Ø) for maintenance of the airlift system in the BFT.

2.2. Maturation of culture water

Maturation of the biological filter and microbial communities of the biofloc was previously performed for 60 days. Tilapia (~ 110 g, storage density of ~ 2 kg m^{-3}) were grown in the BFT and CW systems in three tanks (400 L) each and fed a commercial diet (32% crude protein - CP), at 2% of biomass. The addition of powder molasses in the BFT (C:N ratio of 17:1) and the presence of the filtration system (bag filter (Perlon®) and biological filter with PET caps as substrate for nitrifying bacteria) in the CW were the only factors that differentiated the technologies.

Five days before starting the experiment, 85% of the total volume of the experimental systems were filled with tap water and dechlorinated by strong aeration (for at least 24 h). About two hours before the tilapia juvenile storage, the 15% volume remaining (equivalent to 120 L) was supplemented with the respective inoculum (previously matured waters from each technology). In addition, the PET caps were transferred from the maturation tanks to the CW aquaponic system to compose the biological filter. The choice of inoculating water from both the technologies, contrary to the option of transferring only the inoculum of the bacterial communities of the BFT and the substrates of the biological filter of the CW, aimed to minimize the differences between the initial concentrations of nutrients between the systems. Therefore, the physical-chemical parameters of the CW and BFT inoculums were ammonia: 0.07 and 1.62 mg L^{-1}; nitrite: 0.08 and 0.14 mg L^{-1}; nitrate: 0.00 and 0.02 mg L^{-1}; orthophosphate: 11.45 and 15.80 mg L^{-1}; and alkalinity: 30 and 18 mg L^{-1}, respectively. The settling solids were 8 mL L^{-1} in the BFT.

2.3. Fish

Nile tilapia juveniles (GIFT strain) were purchased from a commercial fish farm and transported in specific bags to the LAQ-UDESC. In the laboratory, the animals were acclimatized for 7 days and fed a commercial diet (36% CP).

In each system, 150 masculinized juveniles (1.14 ± 0.17 g and 4.04 ± 0.23 cm) were stored, totaling an initial biomass of 0.33 kg m^{-3}. Fish were fed a commercial diet (36% CP) four times a day (07:00, 10:00, 14:00 and 18:00) during the experiment. Feed amounts ranged from 12% to 6% of the biomass, adjusted weekly following biometrics performed with 20% of the total number of fish per tank (digital balance, 0.01 g). This range of feed supplied was chosen in order to maximize the performance of fish and plants (Table 1). In this way, a balance was reached between the recommended biomass percentage for tilapia at nursery phase (12% to 8%, based on the manufacturer's recommendation) and the daily amount of nutrient per lettuce (~ 3 g of feed day^{-1} $plant^{-1}$).

At the end of the experiment (day 46) all fish were weighed and the following zootechnical parameters were measured: final weight (g), total length (cm), weight gain (g), productivity (kg m^{-3}), specific growth rate (SGR = [(ln final weight − ln initial weight) × $time^{-1}$] × 100) (% day^{-1}), feed conversion ratio (FCR; amount of feed offered × $gain^{-1}$), survival (%), and viscerosomatic and hepatosomatic indices (viscera or liver weight (s) × $total\ weight^{-1}$).

Table 1. Total amount of feed (kg) offered and its average representations in fish biomass (%) and in daily feed (g) per plant in the aquaponic system using clear-water recirculation (CW) and biofloc technology (BFT), during the two experimental cycles (23 days each).

Treatment	Total amount of feed (kg)	% of fish biomass	g feed day^{-1} $plant^{-1}$
CW - cycle 1	0.96	10%	1.55
CW - cycle 2	2.81	8%	4.52
BFT - cycle 1	1.00	10%	1.61
BFT - cycle 2	2.83	8%	4.56

2.4 Plants

Butter lettuce seedlings (Regina 2000 variety, total weight 3.01 ± 0.59 g, leaf weight 1.68 ± 0.34 g, total length 10.00 ± 0.74 cm, and leaf length 5.52 ± 0.67 cm) were distributed in two production cycles in expanded polystyrene trays at an approximate density of 20 plants m^{-2} (nine seedlings per plant tank). Seedlings were germinated in

specific trays for this phase, with seeds pelletized (Seminis®) and transplanted to the aquaponic system after 35 days of sowing.

At the end of each production cycle, the following phytotechnical parameters were evaluated in all plants: height of leaves (cm), wet and dry weight of leaves and roots (g), number of leaves per plant, yield (kg m^{-2}), and specific foliar growth rate (SFGR = [(ln final leaves wet weight − ln initial leaves wet weight) × $time^{-1}$] × 100) (% day^{-1}). In addition, a plant quality index (PQI) was evaluated by grades attributed to the visual aspect of the leaves. The grades were from A to D as follows: A - excellent, up to 5% of the leaves surface with imperfections; B - good, up to 33% imperfections; C - average, up to 66% imperfections; and D - poor, up to 100% of leaves surface with imperfections. To avoid deviations of interpretation, the PQI evaluation was performed by only one trained evaluator, with a "blind" approach, where the valuator did not know under which technology (BFT or CW) the plants were grown (Pinho et al., 2017).

2.5. Water quality

Water quality parameters including pH (measured using EcoSense pH10A Pen Tester, YSI Incorporated, Yellow Springs, OH, USA), settleable solids (biofloc volume measured in Imhoff cones), temperature, and dissolved oxygen (measured using YSI Mod. 55, YSI Incorporated, Yellow Springs-OH, USA) were monitored daily in the fish and plant tanks of all systems. Concentrations of total ammonia nitrogen (TAN), nitrite (NO_2-N), nitrate (NO_3-N), and orthophosphate (PO_4-P) in all fish tanks were monitored twice a week using photocolorimetric protocols (using Alfakit AT 101, Alfakit, Florianópolis, SC, Brazil). Alkalinity was also measured twice a week, through titration (Alfakit 2460 and 2058, Alfakit, Florianópolis, SC, Brazil).

2.6. Inputs and outputs of nutrients

Samples of feed (20 g), molasses (10.5 g diluted in 210 mL), and supply water (200 mL) were collected before commencing the experiment to characterize their chemical composition. Samples of plants (n = 27 per replicate), whole fish (n = 20 per replicate), and tank water (200 mL per replicate) were collected at three different times: initial, at the end of the first plant cycle (day 23), and at the end of the second plant cycle (day 46). The samples were preserved at -20 °C for subsequent analysis in the Agronomic

Laboratory–UNITHAL (Campinas, SP, Brazil), except for plants that at the end of each cycle were dried at room temperature (37 °C) for 48 h.

The feed, molasses, fish (collected on day 1 of cycle 1 and cycle 2), seedlings, supply water (evaporation loss compensation), and tank water (sampled on day 1 of cycle 1 and cycle 2) were considered items that provided nutrients to the systems. The outputs were determined from the concentration of nutrients in the fish, plants, and tank water (all sampled at the end of cycle 1 and cycle 2), plus the surplus (or other item), calculated by the difference between the inputs and outputs for each nutrient.

All items that provided input and output of nutrients were oven-dried at 500 °C for 16 h to obtain the dry matter data. Concentrations of the major macronutrients and micronutrients required for plant growth (nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), and sodium (Na)) were measured with an Atomic Absorption Spectrophotometer (model 2380, Manasquan, NJ, United States) except for N that was quantified by the Kjeldahl method. Although analyses were performed using the dry matter, all values were transformed to natural or humid matter (Zou et al., 2016).

2.7. Planktonic community

To characterize the planktonic community present in the aquaponics systems using CW and BFT, water samples were collected in triplicate once a week from each fish tank (37 mL). Water samples were also collected in triplicate from the CW and BFT inoculum. A total of 48 samples were fixed in 4% formalin and stained with Rose Bengal for subsequent analysis. For the metazoan count and identification, samples were screened in Petri dishes under a stereo microscope (BIOPTIKA L20T, Bioptika, Colombo-PR, Brazil). For other groups of microorganisms, two 1 mL aliquots were screened in a Sedgewick-Rafter chamber under an optical microscope (Emerenciano et al., 2013b; Pinho et al., 2017).

2.8. Statistical analyses

All data were analyzed for normality (Kolmogorov-Smirnov's test) and homogeneity (Levene's test) of variance (Sokal and Rohlf, 1995). The data of zootechnical performance, water quality parameters, and planktonic community profiles were analyzed with Student's *t*-test. Plant phytotechnical parameters and the PQI were analyzed by two-way analysis of variance (ANOVA) (factors: technology and productive

cycle). Significant differences between the means of treatments were evaluated by Tukey's HSD test at 5% significance (Sokal and Rohlf, 1995).

3. Results

Dissolved oxygen concentrations remained similar between systems during each plant cycle, with mean values of 6.72 ± 0.09 and 6.01 ± 0.25 mg L^{-1} during cycle 1 and cycle 2, respectively, and maximum and minimum values of 7.35 and 4.96 mg L^{-1}. The mean values of water temperature for both systems were 27.66 ± 0.07 °C in cycle 1 and 27.80 ± 0.05 °C in cycle 2, while the maximums and minimums were 29.73 and 25.97 °C, respectively. The value of settleable solids in the BFT fish tanks ranged from 0.0 to 1.2 mL L^{-1}, with a significant difference ($p = 0.029$) between the means of cycle 1 (0.22 ± 0.33 mL L^{-1}) and cycle 2 (0.95 ± 0.18 mL L^{-1}) (Figure 2). The accumulation of floc in the plant tanks (up to 30 mL L^{-1}) was verified; however, it was only possible to measure this after revolving the deposited floc at the end of the experiment. Fluctuations in other parameters measured throughout the experiment are presented in Figure 3. It was observed that the pH, nitrate, alkalinity, and orthophosphate values were higher ($p < 0.05$) in the BFT (mean values of 7.5, 0.23, 98.56, and 4.13 mg L^{-1}, respectively) than in the CW (mean values of 6.6, 0.05, 23.13, and 3.06 mg L^{-1}, respectively) during most of the experimental period. The concentrations of nitrite varied throughout the experiment, with higher values in the CW than in the BFT from the middle of plant cycle 2, whereas ammonia concentration remained higher in the CW than in the BFT from the beginning of cycle 2.

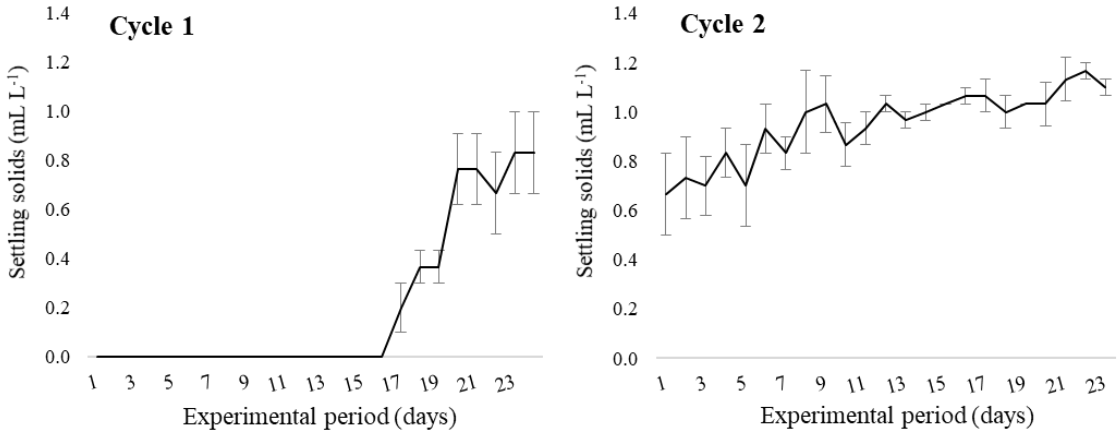


Figure 2. Variation (mean ± standard deviation) in the volumes of settling solids (mL L^{-1}) in the fish tanks of the biofloc technology (BFT), during the two experimental cycles.

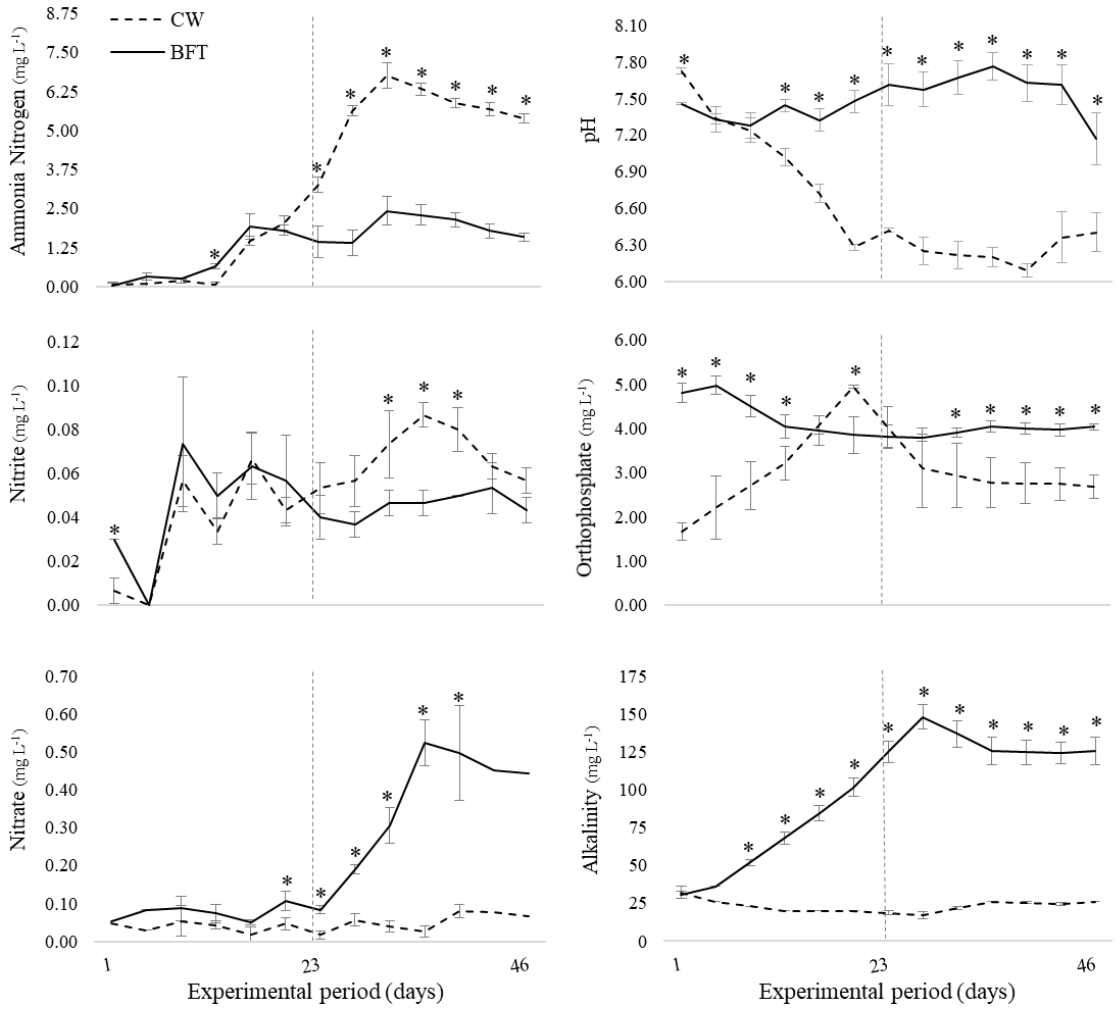


Figure 3. Variation (mean ± standard deviation) in the physical-chemical parameters of water in the aquaponic system using clear-water recirculation (CW) and biofloc technology (BFT), during the two experimental cycles (23 days each). The left side of the dotted line (- - -) represents results from cycle 1 and the right side represents results from cycle 2. * Indicates significant difference between the technologies in each sample time, assessed by the Student's *t*-test at a significance level of 5%.

During the 46 days of the experiment, it was possible to produce Nile tilapia juveniles of 35.77 ± 0.51 g, regardless of the technology used. Except for the productivity, survival, visceral, and hepatosomatic indices, which were statistically equal between the two treatments, the other zootechnical performance parameters were higher ($p < 0.05$) in BFT than in CW (Table 2).

Table 2. Mean (± standard deviation) of productive performance of Nile tilapia juveniles in the aquaponic system using clear-water recirculation (CW) and biofloc technology (BFT) at the end of the experiment (46 days).

Parameters	**CW**	**BFT**	**Value of *p****
Final weight (g)	34.9 ± 0.41[b]	36.69 ± 0.61[a]	0.012
Total length (cm)	12.2 ± 0.04[b]	12.42 ± 0.10[a]	0.016
Weight gain (g)	33.7 ± 0.42[b]	35.56 ± 0.61[a]	0.012
Productivity (kg m^{-3})	7.85 ± 0.11	8.05 ± 0.12	NS
SGR (% day^{-1})	7.43 ± 0.04[b]	7.55 ± 0.05[a]	0.023
FCR	0.88 ± 0.01[b]	0.85 ± 0.01[a]	0.037
Survival (%)	98.8 ± 1.34	96.48 ± 1.78	NS
Viscerosomatic indices	15.9 ± 0.51	15.92 ± 0.41	NS
Hepatosomatic indices	2.5 ± 0.25	2.26 ± 0.19	NS

* Lowercase letters indicate significant difference between technologies for each parameter assessed by the Student's *t*-test at significance level of 5%. SGR: specific growth rate; FCR: feed conversion ratio.

Interaction between the factors (technology and production cycle) was found in all phytotechnical parameters studied ($p < 0.05$) (Table 3). When comparing the cycles in each technology, CW did not affect the performance of the plants between the cycles, whereas in BFT, the lettuce cultivated during cycle 2 had inferior quality compared with that produced during cycle 1. In contrast, when comparing the technologies in each cycle, BFT responded better in cycle 1 than in cycle 2. In cycle 2, except for the wet weight of roots, which were statistically equal ($p > 0.05$) between CW and BFT, other phytotechnical parameters were found to be better in CW than in BFT.

Table 3. Means (± standard deviation) of the productive performance of butter lettuce in the aquaponic system using clear-water recirculation (CW) and biofloc technology (BFT) at the end of each experimental cycle (23 days each), and analysis of the statistical interaction between the factors (technology × productive cycle).

Treatments	**CW - cycle 1**	**CW - cycle 2**	**BFT - cycle 1**	**BFT - cycle 2**	**Value of *p****
Height of leaves (cm)	28.49 ± 1.0^{Ba}	29.19 ± 1.3^{Aa}	30.21 ± 1.2^{Aa}	26.73 ± 2.3^{Bb}	<0.001
Wet weight leaves (g)	145.23 ± 13.2^{Ba}	145.78 ± 14.1^{Aa}	164.70 ± 12.2^{Aa}	100.02 ± 19.0^{Bb}	<0.001
Wet weight roots (g)	13.68 ± 2.1^{Ba}	16.14 ± 3.7^{Aa}	19.82 ± 2.0^{Aa}	17.29 ± 3.0^{Aa}	0.011
Dry weight leaves (g)	5.49 ± 0.5^{Aa}	5.36 ± 0.5^{Aa}	5.99 ± 0.5^{Aa}	3.58 ± 0.8^{Bb}	<0.001
Dry weight roots (g)	0.74 ± 0.1^{Bb}	1.05 ± 0.1^{Aa}	0.96 ± 0.1^{Aa}	0.70 ± 0.1^{Bb}	<0.001
Number of leaves	37.69 ± 1.4^{Aa}	38.28 ± 2.3^{Aa}	36.74 ± 2.8^{Aa}	34.37 ± 1.8^{Bb}	0.047
Yield (kg m^{-2})	2.61 ± 0.2^{Ba}	2.62 ± 0.3^{Aa}	2.96 ± 0.2^{Aa}	1.80 ± 0.3^{Bb}	<0.001
SFGR (% day $^{-1}$)	19.37 ± 0.4^{Ba}	19.14 ± 0.4^{Aa}	19.92 ± 0.3^{Aa}	17.44 ± 0.9^{Bb}	<0.001

* *p* value of the interaction of two-way ANOVA (factors: technology × productive cycle). Capital letters indicate differences between technologies in the same cycle and lowercase letters indicate differences between cycles for the same technology. SFGR = specific foliar growth rate.

The PQI indicated that the visual characteristics also interacted between the technologies and the production cycles (Figure 4), except for the B grades that presented differences only between the cycles, with a higher percentage in cycle 1 than in cycle 2 ($p = 0.018$). In general, in cycle 1 the lettuce presented medium quality, which was similar between the systems. In cycle 2, the lettuce grown in CW showed a significant improvement over cycle 1, whereas visual characteristics declined significantly in BFT from cycle 1 to cycle 2.

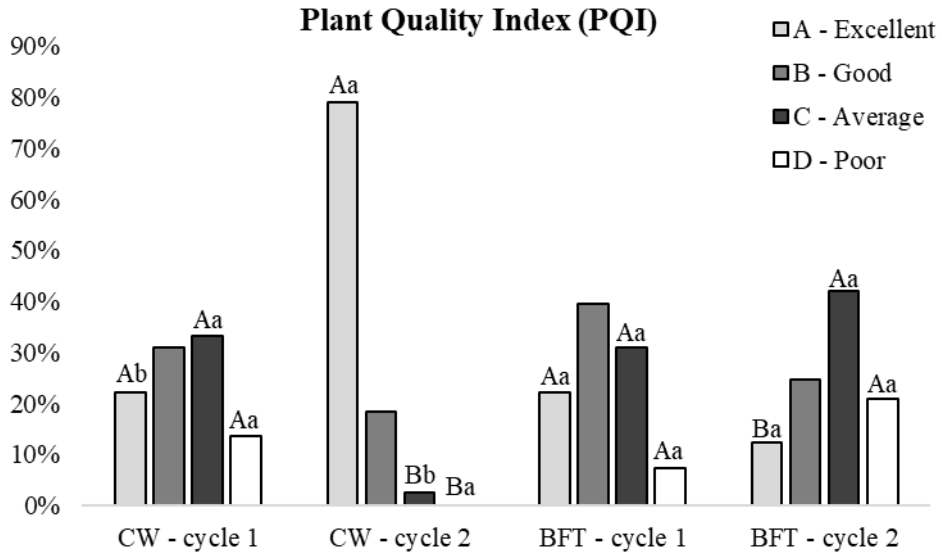


Figure 4. Mean percentage of plants that received grade A – excellent (up to 5% of the leaf surface with imperfections), B – good (up to 33% imperfections), C – average (up to 66% imperfections), and D – poor (100% of leaf surface with imperfections), per treatment. * Letters refer to the interaction by two-way ANOVA (factors: technology × productive cycle). Capital letters indicate differences between the technologies in the same cycle and lowercase letters represent differences between cycles for the same technology.

Regarding to nutrient dynamics, there were differences between the technologies in the amount of nutrient input (g), mainly owing to the addition of molasses in the BFT. Between the two cycles, regardless of the system, there was a 2.5-fold increase in the mean nutrient concentration (Figure 5). In all treatments, plant seedlings represented less than 1% of the total input of all nutrients; therefore, they are not shown in Figure 5.

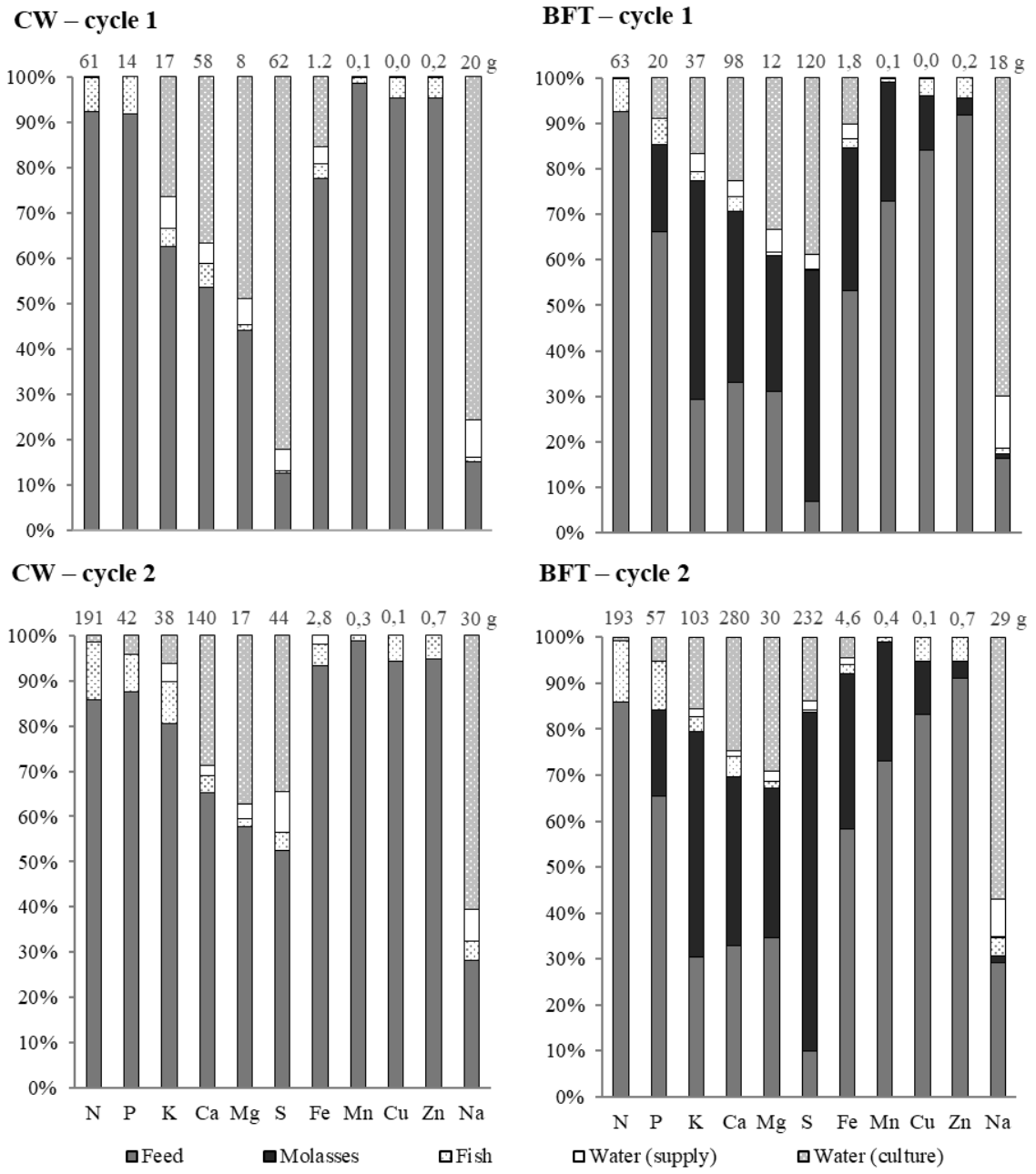


Figure 5. Proportion of the sources and amounts (g) of nutrient inputs in the aquaponic production of butter lettuce and Nile tilapia juveniles using clear-water recirculation (CW) and biofloc technology (BFT), during the two experimental cycles (23 days each).

In contrast, it was observed in the output of nutrients that the proportions between nutrient absorption by different components were numerically similar between the CW and BFT during cycle 1 (Figure 6). However, during cycle 2, the proportions in the CW were similar to that of cycle 1, with greater absorption of nutrients by the plants, whereas

in the BFT in cycle 2, the absorption capacity of the plants was limited, although nutrients were available in water.

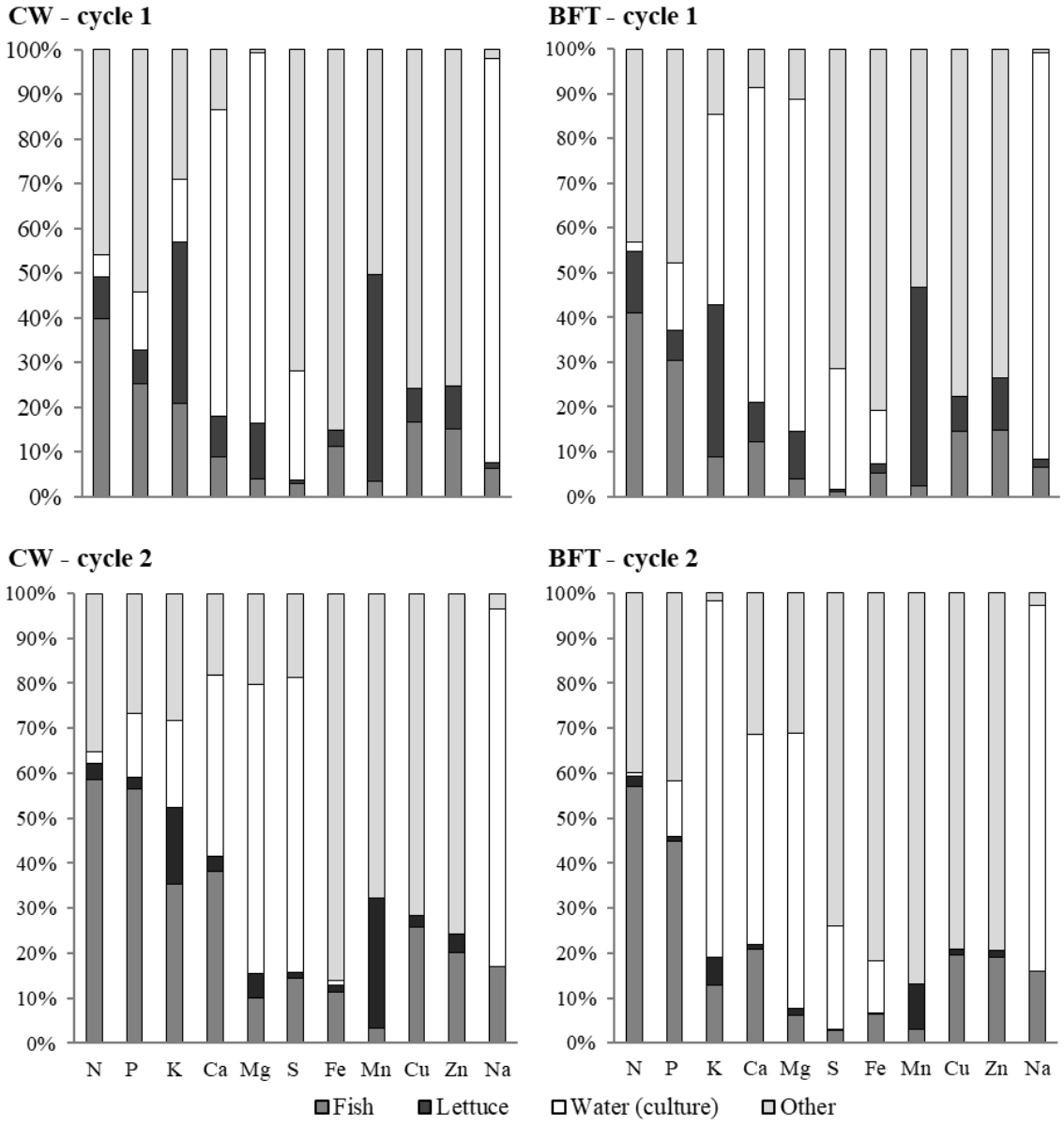


Figure 6. Proportion of compartments that accumulated nutrients in the aquaponic production of butter lettuce and Nile tilapia juveniles using clear-water recirculation (CW) and biofloc technology (BFT), during the two experimental cycles (23 days each).

The characterization of the planktonic community during the 46 days of the experiment revealed six taxa in the CW and eight taxa in the BFT, with microalgae, ciliate protozoa, other protozoans, rotifers, flagellate protozoa, ostracods, cladoceras, and oligochaetes found in decreasing order of abundance. The results indicated a higher frequency ($p < 0.05$) of microalgae in the CW than in the BFT throughout the experiment,

whereas rotifers, flagellate protozoa, and ostracods were statistically more abundant in the BFT than the CW, mainly from the fourth week onwards (Figure 7).

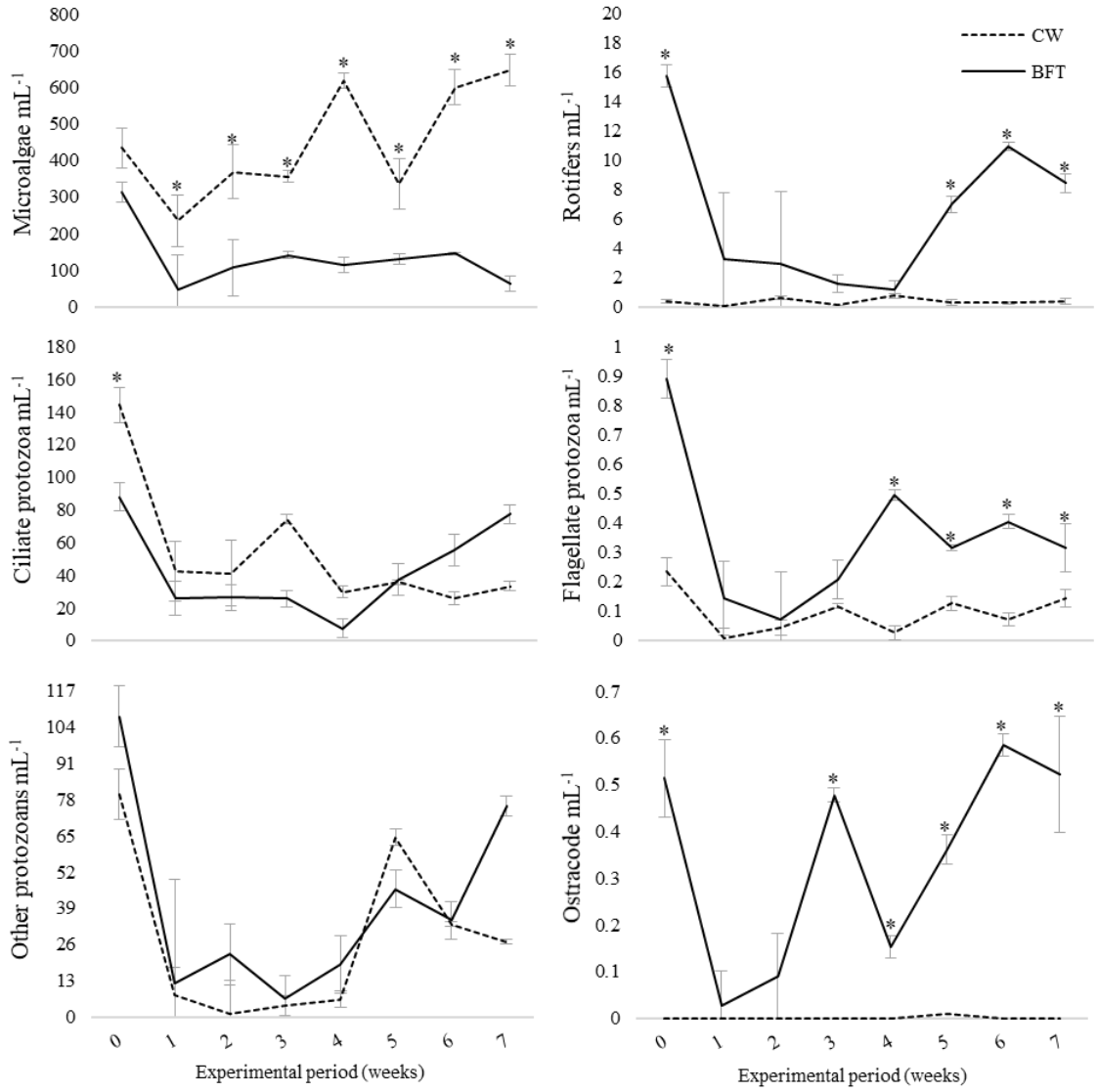


Figure 7. Abundance (organisms mL^{-1}) of planktonic organisms (mean ± standard error) in aquaponics using clear-water recirculation (CW) and biofloc technology (BFT), during the experiment (46 days). * Indicates significant difference between the technologies in each sample time, assessed by the Student's *t*-test at significance level of 5%.

4. Discussion

The differences observed for the water quality parameters of the two technologies were expected, since BFT and CW are different production technologies with distinct physical, chemical, and microbial-complexity characteristics. However, in both systems the recorded values were within the acceptable range for tilapia rearing (El-Sayed, 2006).

Moreover, except for the volume of biofloc (settleable solids) in the BFT and the pH and ammonia concentration in the CW, other values agreed with those verified in tilapia culture in bioflocs (Azim and Little, 2008; Emerenciano et al., 2017; Pinho et al., 2017) and within those normally observed in aquaponics systems (Trang et al., 2017).

The zootechnical results showed that the BFT was more efficient than the CW to produce Nile tilapia juveniles, corroborating the finds observed by Azim and Little (2008) and Luo et al. (2014). The advantage can be attributed to the consumption of the microorganisms present in the BFT (Avnimelech, 2007; 2015). Fish performance observed in the present study was superior to that recorded by other authors in the production of tilapia juveniles in BFT. Brol et al. (2017) and Martins et al. (2017) reared Nile tilapia in BFT with initial weights of approximately 3.1 and 3.7 g, and duration of 42 and 60 days, respectively. These authors found values similar to each other, with FCR, SGRs, and survival approximately 1.2, 5% day^{-1}, and 80%, respectively; yet, these were lower than the results obtained in the present study (0.85, 7.55% day^{-1}, and 96%, respectively). It is reasonable to assume that fish growth in BFT systems could be even better if supplementary feed concentrations (i.e., microbial biofloc) in fish tanks were higher, since the mean volumes of settleable solids (0.22 mL L^{-1} in cycle 1 and 0.95 mL L^{-1} in cycle 2) were much lower than the values between 5 and 20 mL L^{-1} recommended for tilapia juveniles in BFT systems (Emerenciano et al., 2017). These low values can be associated with the accumulation of particulate matter in the hydroponic system, which exposed the inefficiency of the filtration system (clarifier + air lift). In addition, this accumulation possibly contributed to the negative performance of the plants during the second production cycle in BFT.

Although the majority of the zootechnical parameters were lower in CW than those in BFT, the productive performance was better than the data recorded during the nursery stage of tilapia in aquaponics system. Bakhsh and Chopin (2012) cultivated lettuce and Nile tilapia (initial weight of 4 g) for 45 days, and obtained FCR, SGR, and survival of 1.6, 5.2% day^{-1}, and 86%, respectively. These results may be related to several factors, such as favorable environmental conditions, quality of diet, and feed management used in the present study. The concentrations of total ammonia in the water was high from the beginning of cycle 2 (~ 6 mg L^{-1}); however, the pH range recorded during this period indicated that most of the ammonia remained in its ionized form and was non-toxic to fish, that is, it did not harm their development (Wongkiew et al., 2017). Conversely, low pH could have impaired plant production, since a narrow range of 6.5 to 7.0 is

recommended to maximize plant nutrient uptake (Tyson et al., 2004). However, the negative effect on plant performance and PQI was not observed in the CW. These values, different from those recommended for aquaponic production, might be linked to the absence of pH correction during the experiment. Certainly, in a commercial production, this correction must be performed frequently to avoid long-term problems for plant and fish performances.

Contrary to the results observed with fish, the productive performance and visual characteristics of lettuce were negatively affected by the BFT during cycle 2, contradicting the findings presented by Pinho et al. (2017). This shows that, with the experimental design used in the present study, although BFT was efficient during cycle 1, the maturation of the system prevented the development of lettuce. In the CW, there was no effect of the cycle on the phytotechnical results; however, the significant improvement of the visual characteristics during cycle 2 showed the positive effect of the maturation of this system on the plants, contrary to the effect of the BFT. Maturation in the CW is related to a greater tendency to stabilize the nutrient amounts in water and the microbial communities (especially in filtration systems). This would imply, from a commercial point of view, greater relevance to the results from cycle 2, since productions are operated most of the time with stabilized systems. Therefore, when analyzing the plants cultivated during cycle 2 in the CW and BFT, there was a greater difference in performance, with the yield and percentage of plants that received the best grades (A and B) being 2.61 and 1.80 kg m^{-2} and 98% and 37%, respectively. Comparing the phytotechnical results of lettuce during cycle 2 in the CW with the literature (Dediu et al., 2012; Palm et al., 2014; Pinho et al., 2017), the conditions of the present study were found to be satisfactory for plant development. Delaide et al. (2016) cultivated lettuce in a hydroponic and aquaponic system, with and without mineral supplementation, and observed that after 35 days the best treatment (aquaponic with mineral supplementation) resulted in plants with a wet leaf weight of 136.26 g. This result is only higher than plants obtained only during cycle 2 of the BFT, which was the worst treatment in the present study. Thus, in cycle 2 of the BFT, the particularities of the technology tested (e.g., nutrient intake by molasses, microbial community, and presence of bioflocs in the roots) might be the main factors that contributed to the negative effects on plant performance.

Regarding to the analysis of nutrient inputs, it was observed that the feed was the main source of minerals in both systems. In BFT, molasses was used as an external source of carbon; however, it also provided high concentrations of K, Ca, S, and Fe to the system.

When comparing the productive performance of the plants with the nutrient concentrations that entered the system, the results suggest that in cycle 2 in the CW the available nutrients were close in concentration to those required for lettuce production, since it showed the best productive yield and visual characteristics. Nutrient concentrations in aquaponics systems normally differ from those in hydroponics crop cultures, which are frequently nutrient supplemented; however, several authors have reported the same performance of lettuce in aquaponics system without supplementation (the input of nutrients was only by feed as in the CW of the present study) and hydroponics production (Delaide et al., 2016; Pantanella et al., 2012).

Analysis of the output and accumulation of nutrients showed that the proportion of nutrients absorbed by the fish was constant. A significant percentage of surpluses (others) were recorded, which might be associated with the accumulation of sludge in sedimentation systems (Delaide et al., 2017) or biocycling by microorganisms (Zou et al., 2016). The data also suggest that the lettuce grown in BFT was unable to efficiently absorb the nutrients, although the amount increased during cycle 2 owing to fish growth and higher feed supply. This can be explained by the imbalance between the nutrients or, mainly by the presence of bioflocs in the roots. The highest wet weight of roots in cycle 2 of the BFT (Table 2) exposed the accumulation of particles in the roots, which impairs nutrient absorption and can result in rot and death of plants (Rakocy, 2012).

Therefore, for BFT to become an integration option with aquaponics, research investments for system engineering enhancement must be performed. Regarding the accumulation of bioflocs in the roots, several alternatives have the potential to change the scenario reported in the present study. The options include increasing the aeration and water movement in the hydroponic units, adjusting the operation of the clarifiers, and, probably the most promising, the adoption of decoupled aquaponics systems or DAPS. In this last option (DAPS), the production units of plants and fishes are individualized, which enables control and regulation of nutrient concentrations and the optimal environmental conditions for each species (Kloas et al., 2015). In addition, Goddek et al. (2016a; 2016b) showed the potential of adding a digester or mineralizer as a third compartment when using the decoupled system, aiming at recycling the sludge and improving the availability of nutrients to the plants. The use of decoupled systems for aquaponics production with BFT could be an alternative for integration, since there is greater control in the particularities of each compartment and thus better use of particulate organic matter.

The differences between the concentrations and distribution profiles of the planktonic communities confirmed the higher natural productivity in the BFT. In the CW, the higher frequency of microalgae could be attributed to constant exposure to sunlight, nutrient availability (Godoy et al., 2011), and low predation or competition. Although the concentrations of microalgae recorded did not cause damage to the system, large-scale production should not ignore the proliferation of photoautotrophic organisms. This proliferation can cause instability in water quality parameters (Brunson et al., 1994), with sudden daily fluctuations in pH and consequent influence on the amount of toxic ammonia to fish or unavailable to plants.

In BFT, the higher frequency of protozoa and rotifers has also been reported in other studies with tilapia culture using this technology (Brol et al., 2017; Martínez-Córdova et al., 2014; Monroy-Dosta et al., 2013). However, Azim and Little (2008) and Monroy-Dosta et al. (2013) observed frequencies of planktonic organisms in the order of 10^6 L^{-1} when rearing tilapia in BFT without integration with aquaponics. The absence of mechanisms adapted to plant production (i.e., frequent use of clarifiers) can justify this value as being ten times higher than that recorded in the present study. In contrast, Pinho et al. (2017) recorded concentrations of organisms in the order of 10^3 L^{-1} (versus a mean of 10^5 L^{-1} in the present study) in lettuce cultivation using the effluent of tilapia culture (weight ~ 70 g) in BFT. These authors also used two serial clarifiers in order to prevent the biofloc moving to the hydroponic units; however, the lower concentrations of planktonic communities observed by the authors might have been due to the absence of an adapted air lift at the bottom of each clarifier or by the adoption of a biological filter, which probably retained even more microbial flocs. These differences in concentrations of planktonic communities and experimental designs between the present study and the cited works (Azim and Little, 2008; Monroy-Dosta et al., 2013; Pinho et al., 2017) highlight the need to improve the retention or deviation of the bioflocs, thereby improving the availability of microorganisms (i.e., complementary food items) to fish, and to make integration with aquaponics more efficient.

Microorganisms in the BFT aid the cycling of nutrients by promoting the microbial loop (Emerenciano et al., 2017; Martínez-Córdova et al., 2014). These microorganisms directly (e.g., heterotrophic bacteria and autotrophic microorganisms) or indirectly (e.g., trophic chain) require nutrients, mainly N and P, to grow (Martínez-Córdova et al., 2016; Ramos-Rodríguez and Conde-Porcuna, 2003). In the present study, the degree of interaction between the BFT microorganisms and plant performance was not measured;

this would be possible with the identification to the species level of the microorganisms to determine their biology, capacity, and requirement for the use of water macro- and micronutrients. More advanced research on the relationship between biofloc microorganisms and the bioavailability of nutrients to plants is required.

Therefore, the results found in the present study demonstrated the optimal adaptation of tilapia in the nursery phase to the proposed intensive system. On the contrary, the production of butter lettuce in aquaponics was less effective during cycle 2 in the BFT. We emphasize that adjustments and adaptations must be tested to make integration possible, especially with regards to system engineering. Innovative technologies (aquaponics + biofloc) can bring great gains to aquaculture in terms of sustainability, diversification of production and resources efficiency. In addition, productive results of the fish in the CW demonstrated that aquaponics production of lettuce and tilapia in the nursery phase could also become a reality. From a commercial point of view, attaching an aquaponics greenhouse to aquaculture farms is a technical option for providing juveniles of adequate size to be stored in growth-out structures and lettuce with added ecological value. Certainly, economic analyses must be undertaken to verify the economic viability of this production.

5. Conclusions

The presence and consumption of biofloc microorganisms provide better results in fish performance in BFT. In contrast, CW aquaponics system provides better visual and butter lettuce production results than the BFT system, with emphasis to the cycle 2, when the system is mature and with more available nutrients. Under the experimental conditions, BFT does not improve the growth and absorption of nutrients by the plants. Therefore, it is concluded that the integration of BFT with aquaponics might be feasible only if mechanisms are developed to refine the critical points of the system required to improve plant production.

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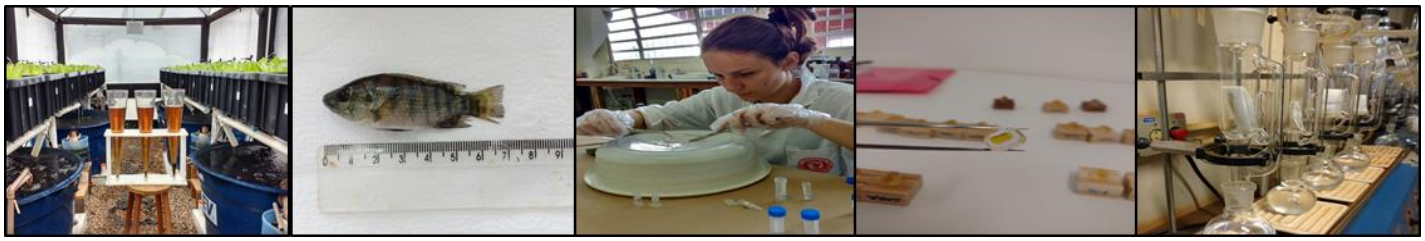
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Artigo 2 - Dissertação Sara Pinho

5. ARTIGO 2

CRESCIMENTO MUSCULAR E COMPOSIÇÃO CORPORAL DE JUVENIS DE TILÁPIA CULTIVADOS EM AQUAPONIA UTILIZANDO A TECNOLOGIA DE BIOFLOCOS

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CRESCIMENTO MUSCULAR E COMPOSIÇÃO CORPORAL DE JUVENIS DE TILÁPIA CULTIVADOS EM AQUAPONIA UTILIZANDO A TECNOLOGIA DE BIOFLOCOS

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Resumo

A obtenção de juvenis de boa qualidade é uma importante etapa para o sucesso da criação de tilápia. Neste sentido, a tecnologia de bioflocos (BFT) parece ser uma alternativa interessante para fornecer juvenis diferenciados para as etapas posteriores de criação. Uma opção para aumentar a eficiência produtiva do BFT é aplicá-lo na produção aquapônica. Desta forma, o objetivo do estudo foi avaliar se, mesmo integrado ao sistema de aquaponia, a tecnologia de bioflocos proporciona melhora na composição corporal e no crescimento das fibras musculares de juvenis de tilápia-do-nilo (*Oreochromis niloticus*), em comparação a um sistema de recirculação de água clara. Para isso, durante 46 dias, juvenis masculinizados de tilápia-do-nilo (1,14 ± 0,17 g), foram cultivados de forma integrada à produção de alface-lisa em sistema aquapônico. Foram avaliados dois tratamentos, com três repetições cada, denominados: recirculação de água clara (CW) ou controle, e bioflocos (BFT). Foram analisadas a composição centesimal e o perfil de ácidos graxos dos peixes e da ração, no início e no final do experimento. Além disso, a morfologia e morfometria das fibras musculares foi verificada quinzenalmente. Os resultados mostraram que não ocorreu diferença estatística entre as tecnologias para todas as análises realizadas, indicando que as condições nutricionais dos peixes foram semelhantes nos dois tratamentos. Isto sugere que a integração com a aquaponia pode ter interferido nos benefícios nutricionais que os bioflocos proporcionam aos peixes, ou que em ambos os tratamentos o manejo alimentar foi adequado e supriu as necessidades dos peixes cultivados.

Palavras chave: nutrição, fibras musculares, alimento natural, berçário.

Introdução

A obtenção de juvenis de boa qualidade para iniciar o processo de engorda é uma importante etapa para o sucesso da criação de tilápia (Ayroza et al. 2011; Barman e Little 2011). Uma estratégia produtiva interessante para isto é o emprego dos berçários ou cultivos multifásicos. A segmentação eficiente da produção está atrelada ao desenvolvimento de sistemas de produção com ênfase na máxima biossegurança, produtividade e mínimo impacto ambiental (Martins et al. 2010). O sistema de bioflocos é uma tecnologia que atende estas premissas (Avnimelech 2015; Emerenciano et al. 2017).

Os cultivos que utilizam tecnologia de bioflocos (BFT) são caracterizados pela troca mínima ou nula de água e o estímulo do crescimento de comunidades microbianas específicas. Os microrganismos do BFT são capazes de incorporar a amônia excretada pelos peixes, fomentando a "alça microbiana" (Martínez-Córdova et al. 2014). Devido a isto, favorecem a manutenção da qualidade da água e servem como fonte de alimento para os animais, *e.g.* juvenis de tilápia, camarões etc. (Azim e Little 2008; Ekasari et al. 2014), diminuindo os valores de conversão alimentar (Luo et al. 2014). Adicionalmente, os agregados microbianos são ricas fontes de nutrientes para os peixes cultivados (Emerenciano et al. 2013). Avnimelech (2007) relatou que os flocos do BFT podem contribuir com aproximadamente 50% da proteína requerida pela tilápia. Long et al. (2015) relataram maior porcentual de lipídeos na carcaça de tilápias (~ 160 g) cultivadas em BFT, em comparação ao sistema controle de água clara, e atribuíram este resultado à disponibilidade de ácidos graxos poli-insaturados (importantes para a nutrição dos organismos aquáticos) nos bioflocos. Desta maneira, a constante oferta e consumo do alimento natural que compõe o BFT pode oferecer condições nutricionais desejadas para a produção de juvenis diferenciados (Bossie e Ekasari 2017).

O acompanhamento do crescimento muscular do animal pode ser uma ferramenta interessante para verificar as condições nutricionais dos juvenis produzidos. Conceitualmente, a musculatura esquelética desenvolve-se por meio de processos de hipertrofia e hiperplasia das fibras (Vélez et al. 2017). Fatores como o regime alimentar, a composição da dieta e as variáveis ambientais podem ocasionar alterações nestes processos, principalmente nos estágios iniciais de desenvolvimento (Leitão et al. 2011; Kojima et al. 2015). Como consequência, modificam o padrão de crescimento das fibras musculares e causam efeitos nas fases posteriores de criação, ou seja, no rendimento

produtivo futuro (Valente et al. 2013). Neste contexto, o maior aporte de alimento natural, como o disponível no BFT, pode proporcionar melhor crescimento muscular quando comparado a outros sistemas de produção, contribuindo para a produção de juvenis diferenciados.

A interação do berçário de tilápia em BFT com a produção de vegetais aquapônicos pode ser uma alternativa para aumentar a eficiência produtiva e, provavelmente, o caráter sustentável da produção de juvenis. Pinho et al. (2017) e Pinheiro et al. (2017) relataram o efeito positivo da utilização dos efluentes do BFT para cultivar diferentes variedades de alface e planta halófica (*Sarcocornia ambigua*), respectivamente. Por outro lado, no artigo 1 foi relatado o efeito negativo do BFT maduro sobre as alfaces cultivadas. Embora os dados produtivos das plantas sejam díspares, em ambos trabalhos os resultados zootécnicos finais dos peixes foram melhores no BFT, em comparação ao sistema de água clara. No entanto, os autores ressaltaram que, devido à necessidade de utilizar constantemente sedimentadores para manter as condições da água requerida para a produção dos vegetais, o volume de floco (indicador da quantidade de floco microbianos) manteve-se baixo ao longo dos experimentos e, como consequência, pode não ter potencializado os benefícios do BFT sobre o desenvolvimento dos peixes. É desconhecido o efeito da redução das concentrações dos agregados microbianos, ao integrar o BFT com a aquaponia, sobre a condição nutricional dos peixes cultivados. Neste sentido, o objetivo deste estudo foi avaliar se, mesmo integrado ao sistema de aquaponia, a tecnologia de bioflocos proporciona melhora na composição nutricional e no crescimento das fibras musculares de juvenis de tilápia-do-nilo, em comparação a um sistema de recirculação de água clara.

Material e Métodos

Desenho experimental

O experimento foi realizado no Laboratório de Aquicultura (LAQ) da Universidade do Estado de Santa Catarina (UDESC), Laguna, SC, Brasil, sob autorização da Comissão de Ética no Uso de Animais (CEUA – Protocolo nº 17615/16). Durante 46 dias, juvenis de tilápia-do-nilo (*Oreochromis niloticus*, 1,14 ± 0,17 g e 4,04 ± 0,23 cm) foram cultivados integrados à produção de alface lisa em sistema aquapônico. Foram avaliados dois tratamentos, com três repetições cada, denominados: água clara (CW) ou controle e bioflocos (BFT), integrado a produção aquapônica. Em ambos os tratamentos foram

estocados, por repetição, 150 juvenis de tilápia recém-masculinizados, totalizando biomassa inicial de 0,33 kg m^{-3} em cada sistema. Os peixes foram alimentados com ração comercial de 36% de proteína bruta - PB, quatro vezes ao dia (07:00, 10:00, 14:00 e 18:00 h). As taxas de alimentação foram ajustadas semanalmente, com biometrias de 20% do número total de peixes, e variaram de 12% nas primeiras semanas até 6% da biomassa no final do experimento. Os resultados detalhados do desempenho zootécnico dos peixes estão apresentados no artigo 1, bem como dos parâmetros físico-químicos da água, do balanço de nutrientes nos sistemas e da caracterização da comunidade planctônica. Na tabela 1 estão resumidos os principais resultados relacionados ao desempenho dos peixes.

Tabela 1. Principais resultados do desempenho de juvenis de tilápia-do-nilo cultivados em sistema aquapônico utilizando a tecnologia de bioflocos (BFT) e recirculação de água clara (CW), durante 46 dias de experimento (compilados do artigo 1).

Parâmetros	**CW**	**BFT**
Peso final (g)	34,9	36,7*
Conversão alimentar	0,88	0,85*
Volume de floco (mL L^{-1})		0 a 1,2
Comunidade planctônica (nº táxons)	6	8
Descrição comunidade planctônica	Maior frequência de microalgas.	Maior frequência de rotíferos, protozoários flagelados e ostracodas.

* Indica diferença estatística entre as tecnologias, pelo teste t-Student a 5% de significância (artigo 1).

Cada réplica experimental foi construída como um sistema independente de recirculação, com as características específicas do tratamento testado. A descrição detalhada do dispositivo experimental pode ser encontrada no artigo 1. Resumidamente, os sistemas com água clara possuíam os compartimentos usuais de um sistema aquapônico, com o tanque de peixes, a bancada hidropônica (tipo bandejas flutuantes, com três tanques de 0,5 m^3), filtros mecânicos e biológicos (Pinho et al. 2017). Enquanto que o de BFT, além do tanque de peixes e os de plantas, possuía dois sedimentadores com "air lift" adaptado. Além disso, foi adicionado melaço em pó como fonte externa de carbono, para manter a relação C:N de 20:1, somente no sistema BFT.

Desempenho peixes

Biometrias semanais foram realizadas para mensurar o peso médio dos peixes (balança digital com precisão de 0,01 g) e verificar a trajetória do crescimento em peso ao longo dos 46 dias experimentais, em cada tratamento. O mínimo de 20% dos peixes de cada repetição (n=30) foram amostrados, com exceção do final do experimento quando todos os peixes foram pesados.

Morfologia e morfometria das fibras musculares

A cada duas semanas, fragmentos de tecido muscular da região epaxial de quatro peixes por repetição (n = 12 por tratamento) foram coletados. Somado a isso, amostras de 12 peixes do lote inicial também foram coletadas, totalizando quatro momentos para análise: dia 0 (inicial); dia 14 (semana 2), dia 28 (semana 4) e dia 46 (final). Esse material foi fixado em solução Karnovsky modificada (glutaraldeído 2,5% + paraformaldeído 2,5%) por 24 horas, lavadas em tampão fosfato de Sorensen 0,1M pH 7,4 e conservadas em etanol 70%, sob refrigeração (~5 ^{o}C). Após esses procedimentos, as amostras foram lavadas em tampão fosfato de Sorensen 0,1 M pH 7,4 e desidratadas em soluções de concentrações crescentes de etanol 80, 90 e 95%, por 1 hora em cada troca. Após a desidratação, as amostras foram incluídas em Historesina® (Leica, Alemanha), seguindo as recomendações do fabricante. Após o emblocamento, foram obtidos cortes histológicos transversais de 3 µm de espessura e corados com Hematoxilina de Harris e Floxina B 0,5% (HF).

A área total, de no mínimo 1000 fibras musculares brancas de cada amostra, foi mensurada por meio de sistema de análise de imagens (CellSens Standart 1.8), e o diâmetro (D) de cada fibra foi determinado utilizando a seguinte fórmula: $D = 2\ A^{0.5}\ \pi^{-0.5}$, onde A = Área total da fibra muscular. Após a mensuração, as fibras musculares foram distribuídas em classes, de acordo com seu diâmetro (µm): classe 10 = $d \leq 10$; classe 20 = $10 < d \leq 20$; classe 30 = $20 < d \leq 30$; classe 40 = $30 < d \leq 40$, classe 50 = $40 < d \leq 50$ e classe 60 = $d > 50$ para avaliação do grau de hipertrofia (> 50 µm) e hiperplasia (< 20 µm) das fibras, adaptado de Assis et al. (2004) e Almeida et al. (2008).

Composição centesimal dos peixes e da ração

Para determinação das composições da ração e corporal dos peixes, foram utilizados trinta indivíduos da amostra inicial, trinta de cada repetição por tratamento no final do experimento e aproximadamente 10 g de ração. Os peixes foram anestesiados em bezocaína e eutanasiados por perfuração cranial, em seguida foram acondicionados com as vísceras e congelados em freezer para posterior análise. Os peixes foram moídos congelados, homogeneizados em moedor de carne (C.A.F, modelo 22S) e liofilizados para então serem separadas amostras destinadas à determinação da porcentagem de matéria seca, proteína bruta (Leco Nitrogen/Protein in Organic Samples, modelo FP528), extrato etéreo (pelo método de hidrólise ácida), matéria mineral e energia bruta (bomba calorimétrica de Parr), de acordo com a metodologia da A.O.A.C. (2000). As mesmas análises foram aplicadas para verificar a composição da ração.

Os resultados obtidos nas análises de composição centesimal foram utilizados para calcular a eficiência de retenção de proteína bruta e de energia bruta, além das proporções de proteína bruta e de extrato etéreo no ganho em peso, de acordo com as seguintes equações: Eficiência de retenção de proteína bruta (ERPB%) = [(PBf x Pf) – (PBi – Pi)] x CPB^{-1}; Eficiência de retenção de energia bruta (EREB%) = [(EBf x Ef) – (EBi x Ei)] x CEB^{-1}; Proporção de proteína bruta no ganho de peso (PBgp%)= [(PBf x Pf) – (PBi x Pi)] x $(Pf – Pi)^{-1}$; Proporção de extrato etéreo no ganho de peso (EEgp%)= [(EBf x Ef) – (PEi x Ei)] x $(Pf – Pi)^{-1}$. Onde PBf, EBf e EEf referem-se aos níveis de proteína bruta, energia bruta e extrato etéreo final corporal; PBi, EBi e EEi aos níveis de proteína bruta, energia bruta e extrato etéreo da amostra inicial dos peixes; CPB e CEB ao consumo de proteína bruta e energia bruta; Pi e Pf ao peso médio inicial e final dos peixes.

Perfil de ácidos graxos dos peixes e ração

Os lipídios totais da carcaça dos peixes já liofilizados (mesmas amostras preparadas na avaliação da composição centesimal) foram extraídos pelo método de Folch et al. (1957), utilizando clorofórmio: metanol e água (2:1,0:0,5). O extrato foi metilado com cloreto de acetila (HCl a 5% em metanol) e convertidos em éster metílico de ácido graxo (FAME). A análise dos ácidos graxos (AG) foi realizada em um cromatógrafo a gás Varian (GC, Modelo 3900, Walnut Creek, CA, EUA), com detector de ionização de chama (FID) e um autoinjetor automático (CP-8410). Os FAMEs foram analisados em uma coluna capilar (CP Wx 52 CB, 0,25 μm de espessura, 0,25 mm de diâmetro interno e 30 m de comprimento). O hidrogênio foi utilizado como gás de arraste, a uma

velocidade linear de 22 cm s^{-1}. A coluna foi programada a 170 °C durante 1 min, seguido de uma rampa de 2,5 °C min^{-1} a 240 °C e um tempo de espera final de 5 min. As temperaturas do injetor e FID foram de 250 e 260 °C, respectivamente. Os ácidos graxos foram identificados comparando-se seus tempos de retenção com aqueles obtidos com padrões comerciais (Supelco, 37 components; Sigma–Aldrich; Mixture, Me93, Larodan and Qualmix, PUFA fish M, Menhaden Oil, Larodan). Os dados foram apresentados como porcentagem de FAME total com base na análise das áreas de pico.

Análises estatísticas

Todos os dados foram analisados quanto à normalidade (Kolmogorov-Smirnov's test) e homogeneidade (Levene's test) de variâncias (Sokal e Rohlf 1995). Os dados das frequências das fibras musculares de cada classe e das porcentagens de ácidos graxos (AG) foram transformados em arco seno e submetidos ao teste t-Student. Análises descritivas foram realizadas para determinar os grupos de AG predominantes entre os tratamentos, divididos em saturados (SFA), monoinsaturados (MUFA), poli-insaturados (PUFAS) n-3, n-6 e totais, e a relação n-3/n-6. Teste *t*-Student também foi aplicado para avaliar os resultados da composição corporal e dos parâmetros de eficiência nutricional dos peixes. As trajetórias de crescimento em peso dos peixes foram linearizadas ($\log_{10}(x-1)$) e submetidos a análise de covariância (ANCOVA) para testar a homogeneidade dos ângulos das curvas de crescimento dos peixes entre os tratamentos CW e BFT. Todos os dados foram analisados a 5% de significância.

Resultados

As trajetórias de crescimento em peso dos peixes apresentaram regressão polinomial (Figura 1) em ambos os tratamentos. Após a linearização, a análise de covariância mostrou não haver diferença (ANCOVA, p = 0,695) entre os ângulos das curvas de crescimento dos peixes cultivados na CW ou BFT, demonstrando que os peixes apresentaram taxas de crescimento similares entre os tratamentos.

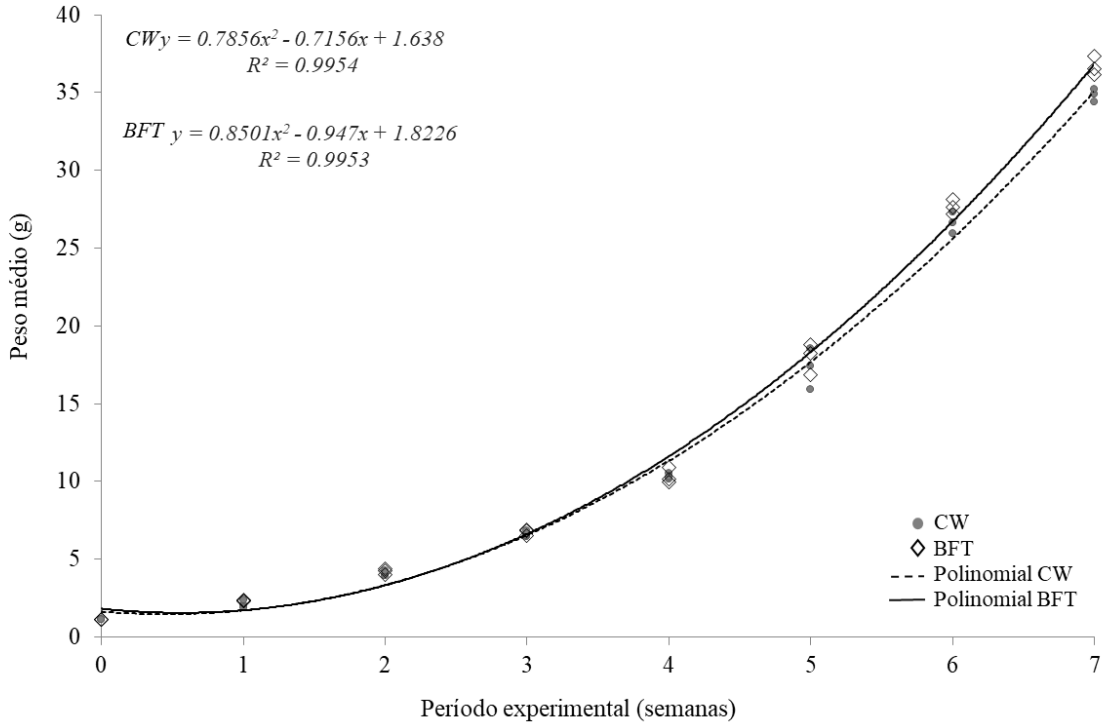


Figura 1. Curva de crescimento de juvenis de tilapia-do-nilo cultivados em sistema aquapônico utilizando a tecnologia de bioflocos (BFT) e recirculação de água clara (CW), durante 46 dias de experimento (amostragem semanal, n=30).

As análises da composição corporal e dos parâmetros de eficiência nutricional mostraram que não houve diferença estatística entre os peixes cultivados em CW e BFT (Tabela 2). Na tabela 2 está também apresentada a composição centesimal da ração ofertada e dos peixes antes de iniciar o experimento. O perfil de ácidos graxos (AG) da ração e da carcaça dos peixes, no início e final do experimento, estão apresentadas na tabela 3. Não ocorreu efeito ($p > 0,05$) dos tratamentos sobre o perfil de AG.

Tabela 2. Composição centesimal da ração e da carcaça, bem como parâmetros de eficiência nutricional dos juvenis de tilápia-do-nilo cultivados em aquaponia utilizando tecnologia de bioflocos (BFT) e recirculação de água clara (CW), após 46 dias de experimento.

Parâmetros	**Ração**	**Inicial**	**CW**			**BFT**			**Valor de *p****
Composição									
PB (%)	36,56	16,93	17,84	±	1,42	17,09	±	0,44	0,43
EB (kcal kg^{-1})	4752,00	1248,94	1442,79	±	77,39	1469,73	±	64,34	0,67
EE (%)	6,61	3,32	6,28	±	0,62	7,03	±	0,17	0,11
MM (%)	13,00	4,94	4,10	±	0,18	4,12	±	0,37	0,93
UM (%)	4,78	74,92	71,46	±	0,36	72,00	±	0,68	0,29
Eficiência									
ERPB (%)			50,24	±	4,58	49,77	±	1,45	0,87
EREB (%)			34,66	±	1,91	36,55	±	1,64	0,26
PBgp (%)			16,16	±	1,47	15,47	±	0,45	0,48
EEgp (%)			6,37	±	0.65	7,15	±	0,17	0,12

* Indica diferença estatística entre as tecnologias pelo teste *t*-Student a 5% de significância. PB: proteína bruta, EB: energia bruta, EE: extrato etéreo, MM: matéria mineral, UM: umidade, ERPB: eficiência de retenção de PB, EREB: eficiência de retenção de EB, PBgp: proporção de PB no ganho de peso, EEgp: proporção de EE no ganho de peso.

Tabela 3. Perfil de ácidos graxos totais (%) (± desvio padrão) da ração e de juvenis de tilápia-do-nilo no início e final do cultivo em sistema aquapônico utilizando tecnologia de bioflocos (BFT) e recirculação de água clara (CW), após 46 dias de experimento.

AG (%)	**Ração**	**Inicial**	**CW**	**BFT**	**Valor de *p****
C14:0	1,55	3,38	3,03 ± 0,15	3,05 ± 0,33	0,98
C16:0	23,58	27,67	29,55 ± 0,14	29,30 ± 1,11	0,70
C18:0	8,89	8,65	8,18 ± 0,07	8,05 ± 0,29	0,50
∑ SFA	34,01	39,70	40,75 ± 14,06	40,40 ± 13,94	0,68
C16:1 n-7	1,78	7,51	4,47 ± 0,12	4,66 ± 0,27	0,31
C18:1 n-9	31,60	31,07	37,26 ± 0,52	36,74 ± 0,84	0,41
C18:1 n-7	1,76	4,44	2,73 ± 0,11	2,80 ± 0,05	0,31
C20:1 n-9	0,54	1,83	2,01 ± 0,11	2,00 ± 0,08	0,92
∑ MUFA	35,67	44,85	46,47 ± 17,13	46,22 ± 16,83	0,69
C18:3n-3	1,88	0,56	0,30 ± 0,03	0,29 ± 0,03	0,75
C21:5n-3	0,36	0,10	0,47 ± 0,01	0,62 ± 0,18	0,22
C22:5n-3	0,19	0,55	0,06 ± 0,01	0,23 ± 0,30	0,82
C22:6n3	NF	2,11	NF ± NF	0,05 ± **	0,38
∑ PUFA n-3	2,44	1,20	0,83 ± 0,21	1,19 ± 0,24	0,34
C18:2 n-6	27,34	8,56	10,12 ± 0,43	9,82 ± 0,59	0,51
C18:3 n-6	0,10	0,50	0,39 ± 0,02	0,36 ± 0,02	0,06
C20:2 n-6	0,10	0,67	0,55 ± 0,03	0,81 ± 0,51	0,45
C20:3 n-6	0,06	0,45	0,37 ± 0,07	0,27 ± 0,04	0,09
C20:4 n-6	0,11	1,01	0,21 ± 0,02	0,18 ± 0,01	0,13
C22:2 n-6	0,08	0,07	0,08 ± 0,02	0,26 ± 0,18	0,15
C22:4 n-6	0,03	0,40	0,14 ± 0,02	0,35 ± 0,33	0,32
C22:5 n-6	0,06	0,47	0,11 ± 0,02	0,18 ± 0,07	0,19
∑ PUFA n-6	27,88	12,13	11,95 ± 3,49	12,23 ± 3,36	0,88
n3/n6	0,09	0,10	0,07 ± 0,06	0,10 ± 0,07	0,25
Σ PUFA total	30,32	13,34	12,78 ± 2,97	13,42 ± 2,86	0,75

* Indica diferença estatística entre as tecnologias pelo teste *t*-Student a 5% de significância. AG: ácidos graxos; SFA: ácidos graxos saturados; MUFA: ácidos graxos monoinsaturados; PUFA: ácidos graxos poli-insaturados; NF: não identificado; ** sem desvio padrão, ácido graxo encontrado apenas em uma repetição.

O crescimento das fibras musculares não apresentou diferença ($p > 0,05$) entre as tecnologias para todas as classes de fibras em cada tempo analisado, com exceção das fibras de classe 10 no 14º e 28º dia. Na figura 2 é possível observar o padrão de ocorrência das diferentes classes em cada tratamento e período de análise, com maior frequência de fibras da classe 20, 30 e 40 no 14º dia, 30 e 40 no 24º dia e 60 no final (46º dia) do período experimental. O padrão morfológico visualizado no material amostrado está ilustrado na figura 3. Em ambos os tratamentos e período amostral foi possível observar células com núcleos periféricos bem distribuídos, inserção normal das fibras no tecido conjuntivo e distribuição em moisaco, com fibras menores entre as de maior diâmetro.

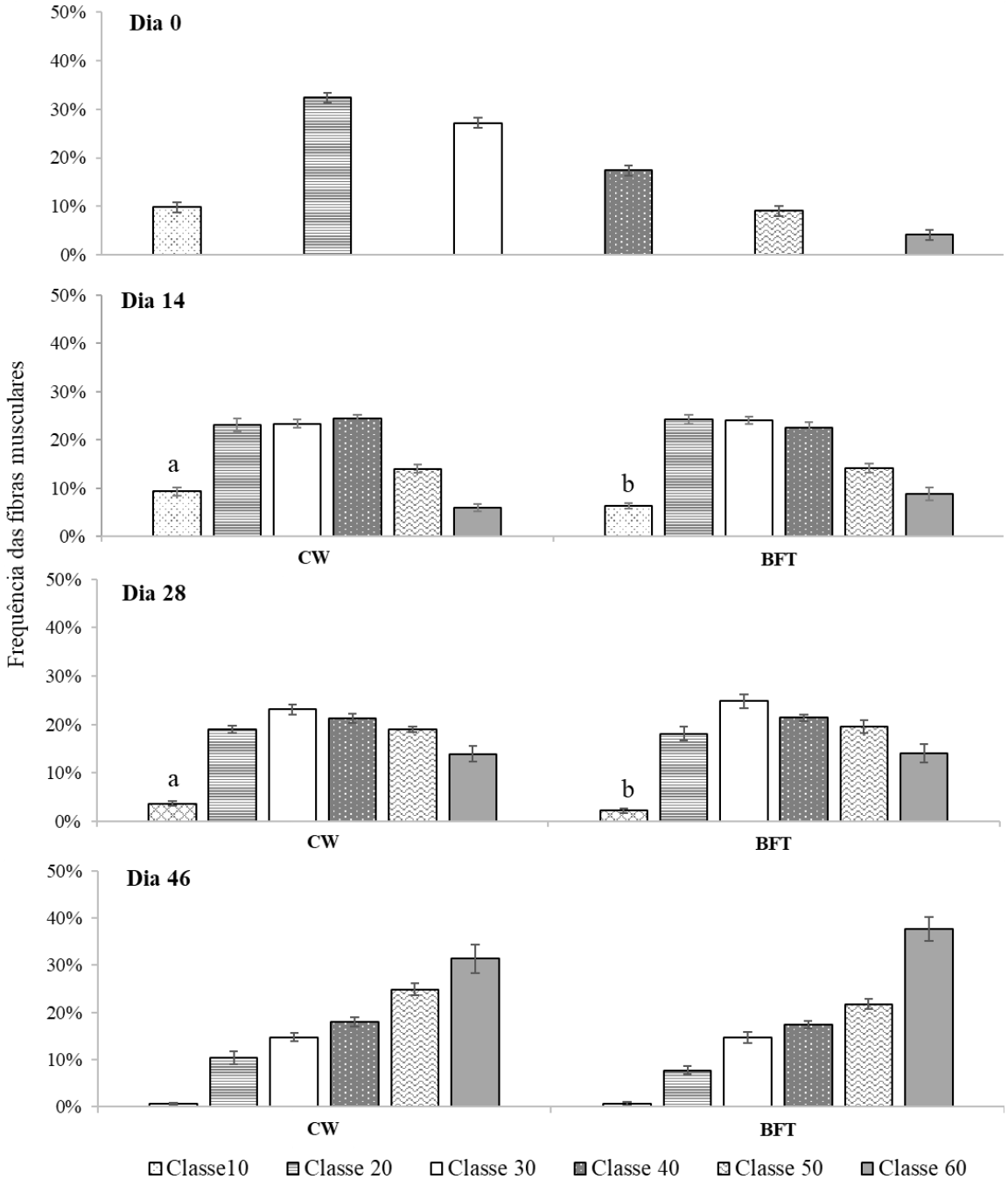


Figura 2. Frequência de classes de diâmetro das fibras musculares de juvenis de tilápia-do-nilo (n=12 por tratamento) cultivados em aquaponia utilizando tecnologia de bioflocos (BFT) e recirculação de água clara (CW), nos diferentes períodos amostrais. * Letras minúsculas indicam diferença estatística entre as tecnologias para cada classe de fibra nos diferentes períodos amostrais, pelo teste *t*-Student a 5% de significância.

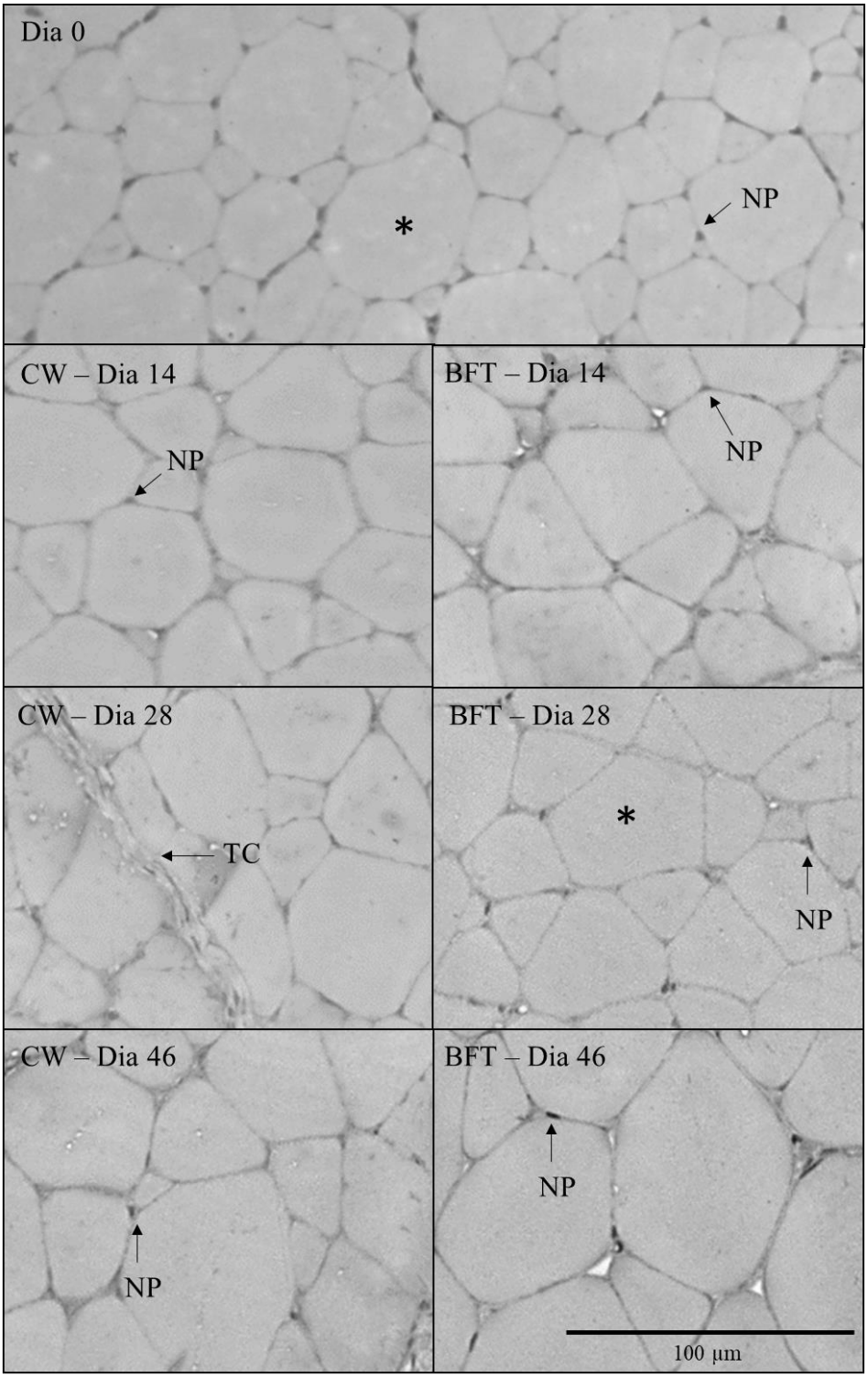


Figura 3. Seções transversais do músculo esquelético de juvenis de tilápia cultivados em aquaponia utilizando tecnologia de bioflocos (BFT) e recirculação de água clara (CW), nos diferentes períodos amostrais. Abreviações: NC – Núcleo periférico; TC – Tecido conjuntivo. * Fibras de maior diâmetro cercadas por fibras menores. Hematoxilina-Floxina B.

Discussão

Os resultados das trajetórias de crescimento em peso dos peixes mostraram que, em ambos tratamentos, os peixes apresentaram taxas de crescimento similares. O peso final médio dos juvenis de tilápia foi estatisticamente maior no BFT (36,7 g) em comparação a CW (34,8 g) (tabela 2), por causa disso esperava-se que os peixes cultivados em bioflocos apresentassem uma trajetória de crescimento mais acelerada. A literatura disponível normalmente apresenta a análise comparativa entre médias finais para constatar os resultados de crescimento em peso. Azim e Little (2008), Luo et al. (2014) e Long et al. (2015) avaliaram o cultivo de tilápia em bioflocos e água clara e observaram peso final superior no BFT, de aproximadamente 140, 168 e 160 g, em relação aos 127, 138 e 146 g da CW, respectivamente. No entanto, a análise da curva de crescimento parece ser também uma abordagem interessante na produção de juvenis de ~ 30 g. Isto porque, diferente da engorda, onde 8% a mais de peso final (artigo 1) pode interferir em fatores importantes de mercado (e.g. rendimento de filé), nos berçários essa variação é considerada pequena (1,4 g) e certamente não interferirá no valor final dos juvenis em uma situação real de cultivo.

O consumo da microbiota do BFT é frequentemente relatado como fator que proporciona diminuição da conversão alimentar (Luo et al. 2014, Pinho et al. 2017) e altera as características centesimais dos organismos cultivados utilizando a tecnologia de bioflocos (Izquierdo et al. 2006; Xu e Pan 2012; Long et al. 2015). No entanto, os resultados do presente estudo mostraram que o consumo não foi suficiente para alterar a composição centesimal e os parâmetros de eficiência nutricional dos juvenis de tilápias cultivados em BFT integrado à produção aquapônica. Além disso, o perfil dos ácidos graxos (AG) também não apresentou diferenças. Nos peixes de ambos os tratamentos foram encontrados maior predominância dos AGs saturados (SFA) (~ 40,5%) e monoinsaturados (MUFA) (~ 46,3%), enquanto que os poli-insaturados (PUFA) corresponderam a aproximadamente 13%, com maiores quantidades de n-6 (~ 12%) em comparação ao n-3 (~ 1%). Embora os PUFAs sejam importantes para o desenvolvimento dos animais e que os peixes sejam reconhecidos como fontes de n-3 para a alimentação humana (Fuchs et al. 2013; Dumbar et al. 2014; Tocher 2015), nas tilápias prevalece maior quantidade dos n-6 em relação aos n-3 (Navarro et al. 2012; Pan et al. 2017). Isto se deve a tilápia ser naturalmente de água doce e ter hábito alimentar onívoro, alimentando-se principalmente de fontes vegetais terrestres (ricos em n-6) (Rasoarahona

et al. 2005; Teoh e Ng 2016). A qualidade da ração ofertada (tabelas 2 e 3) pode ser um dos motivos para justificar a similaridade entre as composições dos peixes na CW e BFT. Isto porque, estudos relacionados às exigências nutricionais das tilápias evidenciam que dietas contendo no mínimo 30% PB e 4% de lipídeos são indicadas para maximizar o crescimento na fase de berçário (Ng e Romano 2013). Além disso, a oferta dos ácidos graxos poli-insaturados, principalmente os ácidos linoléico (18:2n-6) e alfa-linolênico (18:3n-3), encontrados no perfil dos AG da ração (27,3 e 1,88%, respectivamente), são fundamentais para o desenvolvimento das tilápias (Ng e Romano 2013).

Em relação ao crescimento das fibras musculares, de maneira geral, não ocorreu diferença no padrão de crescimento entre os peixes criados em CW ou BFT. Os dados indicaram maior frequência das fibras de classe 10 no 14º e 28º dia na CW, no entanto no final do período experimental este resultado não se refletiu em maior frequência de fibras com diâmetro acima de 50 µm (indicador de hipertrofia). Esperava-se que o BFT, devido ao maior aporte de alimento natural, proporcionasse um incremento das fibras com diâmetro menor que 20 µm (indicador de hiperplasia) no início do desenvolvimento, e que isso refletisse no maior peso corporal final (Dal-Pai et al. 2000). No entanto, o recrutamento de novas fibras parece ter diminuído em momentos similares em ambos tratamentos. A partir de tal momento, o crescimento muscular continuou acontecendo majoritariamente pelo aumento das fibras já existentes (Weathery et al. 1988), fato que fica claro pela visualização do padrão das classes no 46º dia.

Os resultados apresentados contrapõem a hipótese de que o BFT contribui para a melhora das condições nutricionais dos peixes cultivados. Isto porque, todos os parâmetros analisados mostraram que não houve diferença entre os resultados obtidos nos peixes criados em CW e BFT, podendo, no entanto, ser um reflexo de três situações particulares do presente estudo. A primeira, relacionada à baixa quantidade de microrganismos disponíveis na água do BFT, devido às particularidades necessárias para integração entre o BFT e a aquaponia (artigo 1). A segunda, referente ao adequado manejo alimentar empregado, ou seja, a composição da ração, a frequência alimentar e a quantidade de ração ofertada provavelmente foram suficientes para suprir as exigências nutricionais dos peixes. Esse resultado é contrário ao apresentado por Long et al. (2015), que verificaram melhora da taxa de retenção proteica e da porcentagem de lipídeo na carcaça de juvenis de tilápia (~ 50 g) cultivados em BFT, em comparação à CW. Estes autores empregaram um manejo alimentar que provavelmente não potencializou o

crescimento das tilápias na CW, visto que alimentaram os peixes na proporção de 3% da biomassa (versus 12 a 6% no presente estudo); 2 vezes ao dia (versus 4 vezes) e com dietas contendo 46% PB (versus 36% PB). A terceira situação seria a combinação dos dois fatores anteriores, ou seja, a somatória da baixa disponibilidade de alimento complementar com o manejo adequado.

Desta maneira, pressupõe-se que a produção aquapônica para ofertar juvenis de tilápia poderá ocorrer, independentemente da tecnologia empregada (bioflocos ou recirculação em água clara), principalmente se o manejo alimentar adequado for mantido. O benefício do BFT nas condições nutricionais dos organismos aquáticos seria evidenciado em situações específicas, como no uso de baixas quantidades de ração, adoção de frequência alimentar e concentração de proteína da dieta inadequadas (Azim e Little 2008) ou no aumento das densidades de estocagem (Brol et al. 2017). Nos casos em que esses manejos sejam necessários para, por exemplo, diminuir os custos operacionais da produção, a quantidade e a composição dos flocos microbianos devem ser garantidas e suficientes para suplementar a alimentação dos peixes. Nestas situações, o cultivo de juvenis de tilápia em BFT integrado a produção vegetal só seria possível se mecanismos fossem desenvolvidos para manter os microrganismos mais confinados nos tanques dos peixes.

Conclusão

Nas condições experimentais do presente estudo, conclui–se que a fase de berçário de tilápia-do-nilo em BFT, integrado à aquaponia, não apresenta vantagem em relação ao cultivo em CW, haja visto o rápido crescimento dos juvenis em ambos os sistemas, a semelhança das condições nutricionais (composição centesimal e de ácidos graxos na carcaça) e do padrão morfológico e morfométrico das fibras musculares. Isto sugere que, a integração com a aquaponia pode ter interferido nos benefícios nutricionais que os bioflocos potencialmente proporcionariam aos peixes, ou que em ambos os tratamentos (CW e BFT) o manejo alimentar foi adequado e supriu as necessidades dos juvenis.

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Artigo 3 - Dissertação Sara Pinho

6. ARTIGO 3

ECONOMIC COMPARISON BETWEEN CLEAR WATER AND BIOFLOC TECHNOLOGY FOR THE AQUAPONIC PRODUCTION

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ECONOMIC COMPARISON BETWEEN CLEAR WATER AND BIOFLOC TECHNOLOGY FOR THE AQUAPONIC PRODUCTION

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Abstract

Biofloc fish production generally involves a reduced amount of fish feed, continuous cycling of the nutrients, and the possibility of reducing the use of filters, which can lower costs. There is therefore the potential for integrating biofloc technology in aquaponics systems. The present study aimed to compare the economic viability for an aquaponic production of tilapia juveniles and lettuce in a clear-water recirculation system (CW) and in a biofloc technology (BFT). The study was developed as an investment analysis for commercial aquaponic production of juvenile tilapia and butter lettuce. In order to do that, we built four different scenarios, two taking into account the marketable lettuce production: 37 % marketable lettuce from BFTand 98% marketable lettuce from CWof total production. The other two scenarios considered a hypothetical production in which all the lettuce produced have the marketable quality. The economic viability indicators calculated were: Internal Rate of Return (IRR); Net Present Value (NPV); PayBack (PB); Discounted PayBack (DPB); Benefit/Cost ratio (B/C) and Discounted Benefit /Cost ratio (DB / DC). The results showed that the production of juveniles of Nile tilapia and lettuce in CW is profitable, but the 37% marketable lettuce from BFT is not. The break-even point was calculated to know the quantities of plants that should be visually suitable for market, so that the project would be economically profitable. It was estimated that 63.5% of the lettuces produced should have desirable visual marketable characteristics to make the production with BFT feasible.

Keywords: Investment, break-even point, aquaculture, tilapia, lettuce.

1. Introduction

Tilapia is the main species of Brazilian aquaculture. This species represented 47.1% of the volume and 40.9% of the value of the national fish production in 2016, valued around US$ 400 million (IBGE, 2017). Most of the volume is produced in traditional structures of ponds and cages, dominated by single-phase crops, i.e., stocking fingerlings for grow out (Roriz et al., 2017). Sourcing fingerlings from other farms has its problems, which has led to an increasing adoption of nurseries or multiphase culture in aquaculture (Barman and Little, 2011; Nasr-Allah et al., 2014). To some farmers, the multi-phase practice ensures disease control and increases productive efficiency. Subtropical climate producers are also able to manage lower winter temperatures through better culture management. The adoption of nurseries allows the supply of larger juveniles (~ 30 g) that can be more resistant to diseases, and suitable for transfer to grow out structures (Little et al., 2003). In addition, it is attractive for the producer to invest in structures and technologies to segment production, given the rapid capital turnover and low feed utilization in the nursery compared to the growth phase.

A strategy that can make nurseries more economically and environmentally efficient is by implementing them in integration with hydroponic plant cultivation through aquaponics. This is because it is possible to reach high fish and plant productivity, and ensure aggregate ecological value (Edwards, 2015; Yildiz et al., 2017) due to the low formation and recycling of the residues, the non-use of chemicals, and the best use of water and space (Goddek et al., 2015). In addition, the plants considerably increase production revenues. Tokunaga et al. (2015) reported an approximately 80% contribution of plants in total revenue, in an aquaponic production of tilapia and lettuce using floating system in Honolulu, Hawaii, USA. Quagrainie et al. (2017) analyzed different scales of aquaponic farms in the U. S. Midwest and found that plants accounted for 80% of the production volume, regardless of size and level of investment. However, the system requires a high investment compared to the traditional aquaculture structures, especially with the addition of hydroponic beds and filtration systems (Tokunaga et al., 2015; Bosma et al., 2017). These filters, mechanical and biological, characteristic of recirculation intensive aquaculture production, are fundamental for transforming fish residues into nutrients for plant growth (Ru et al., 2017).

Recent studies show the potential of integrating aquaponics with biofloc technology (BFT) for marine shrimps (Pinheiro et al., 2017) and tilapia (Pinho et al., 2017a).

Conceptually, the biofloc system is based on the growth of specific microbial communities, which replace the use of biological filters, through the biological cycling of nutrients (Emerenciano et al., 2017). Biofloc production generally involves a reduced amount of fish feed, continuous cycling of the nutrients, and the possibility of reducing the use of filters. These factors can lower costs and make integration in aquaponics economically profitable. This system also is used to reduce feed conversion factors, to increase productivity and to ensure system biosafety (Crab et al., 2012; Ahmad et al., 2017). Luo et al. (2014) obtained a 22% increase in final weight and reduction in feed conversion of 18% in juveniles of tilapia produced in BFT, compared to the recirculation system in clear-water.

Although the integration between aquaponics and BFT is theoretically attractive, in Article 1 it was showed that 63% of the plants grown in mature biofloc presented undesirable visual characteristics for the market. From a commercial point of view, decision-making about investing in modern technologies will depend on the economic viability of the enterprise (Kodama et al., 2011, Karim et al., 2015). The alternative of increasing diversification and sustainable character of aquaculture production through the application of modern technologies for integration with hydroponic, only will be attractive if it generates profit to the producer. This highlights the importance of verifying whether the integration is cost-effective, and the visual quality of the plants that will make it profitable. Thus, the objective of the present study was to compare the economic viability for the aquaponic production of tilapia juveniles and lettuce in a clear-water recirculation system and in a biofloc technology.

2. Material and methods

The study is an investment analysis for a hypothetical commercial aquaponic production of Nile tilapia juveniles with lettuce (variety butter, subtype Regina 2000). All prices used in the calculation were observed in September 2017 in Sao Paulo State, Brazil, and converted to dollar (quotation in September: US$ 1 = R$ 3.15). The data source of the present analysis was obtained in an experimental research (presented in Article 1 and summarized in Table 1). In the experimental study, the use of the clear-water system in recirculation (CW) and the biofloc technology (BFT) to produce one cycle of tilapia nursery (46 days) integrated with two consecutive cycles of lettuce production (23 days each) were evaluated. In addition to the productive parameters, a Plant Quality Index -

PQI (Pinho et al., 2017a) was evaluated by grades attributed to the visual aspect of the leaves. The grades were from A to D as follows: 'A,' excellent, up to 5% of the leaves surface with imperfections; 'B,' good, up to 33% imperfections; 'C,' average, up to 66% imperfections; and 'D,' poor, up to 100% of leaves surface with imperfections.

Table 1. Summary of the experimental results and basic design used to size aquaponic production of Nile tilapia juvenile and lettuce, using biofloc technology (BFT) and clear-water recirculation (CW).

Experimental design		
Fish cycle (days)	46	
Plant cycle (days)	23	
Fish density (fish m^{-3})	300	
Plant density (plants m^{-2})	20	
Volume ratio (m^3 fish : m^2 plant)	1 m^3 : 3 m^2	
Tilapia results		
	BFT	CW
Survival	98%	98%
Feed conversion rate	0,85	0,88
Lettuce results		
	BFT	CW
Plant Quality Index % marketable plants (A + B)	37%	98%

In the present study, the economic analysis was performed considering the productive data of the fish and only the data of the second cycle of the plants, assuming that in the first the system was still in the maturation phase and the results from the second cycle would be more consistent with a productive reality. The BFT and clear-water recirculation (CW) were analyzed with emphasis on the percentage of plants that received grades A and B in the PQI, since only these were visually suitable for marketing. Thus, four different scenarios were considered, two with the percentages found in experimental culture – BFT: 37% and CW: 98%; and two others, considering a hypothetical production where 100% of the lettuce produced could be sold, called: BFT: 100% and CW: 100%.

2.1. Layout of production

Production planning focused on the supply of juveniles of 30 g Nile tilapia to a medium-sized cages farm, that is, with 150 cages of 6 m³ each, to be populated with approximately 240 thousand juveniles per year, or 20 thousand per month (~ 11 cages populated per month). To reach this demand, in both scenarios it was necessary a single system that interconnected 10 tanks (12 m³ each) for fish production and five hydroponic beds of 72 m each (0.3 x 3.0 x 24.0 m), all built with geomembrane HDPE (High-density polyethylene) and galvanized pop fence (Figure 1). The adopted model was of the floating type, using floating trays of expanded industrial polystyrene of high density.

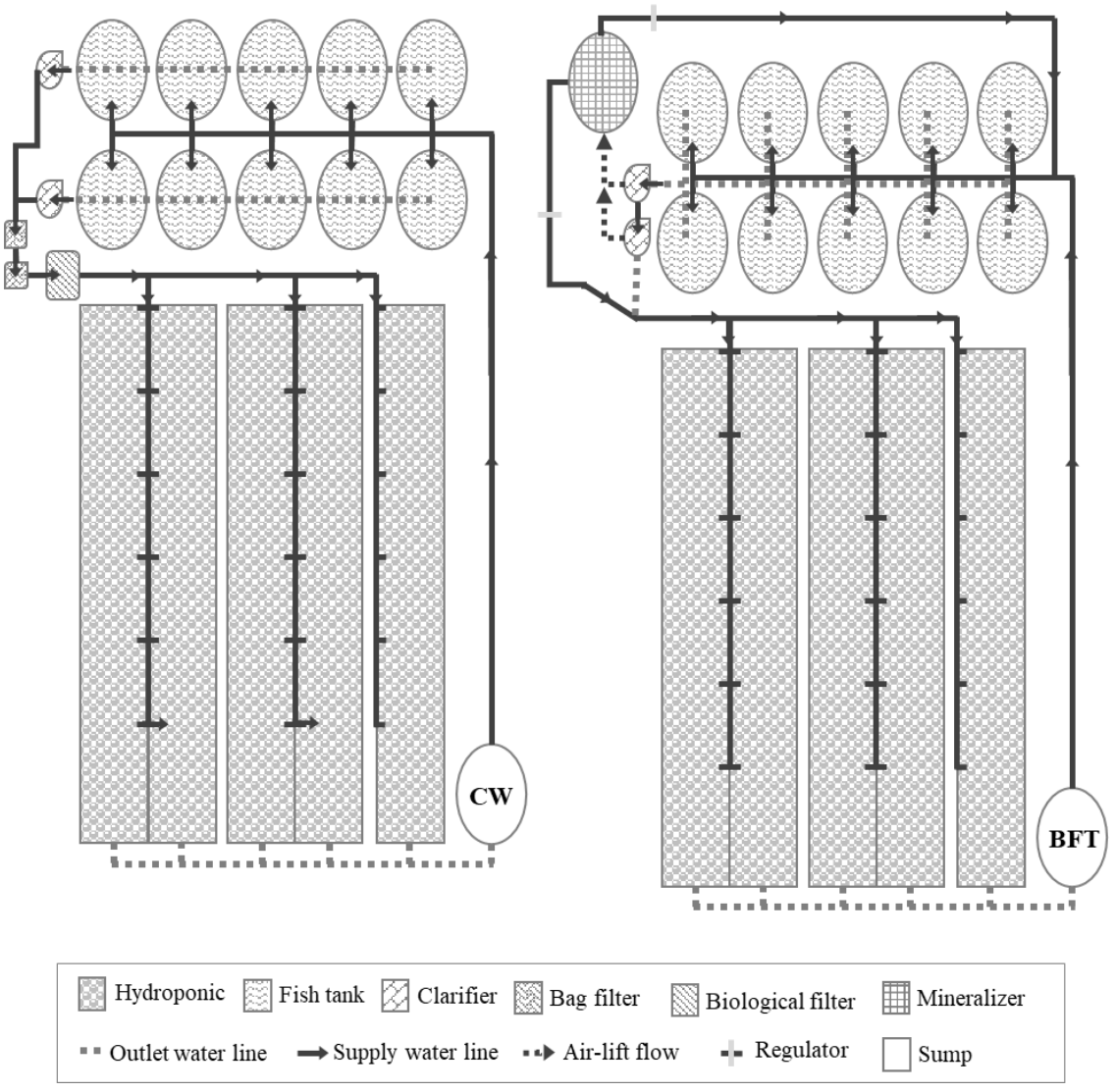


Figure 1. Layout of systems for aquaponic production of Nile tilapia juvenile and lettuce, using biofloc technology (BFT) and clear-water recirculation (CW), sized to produce approximately 280,000 juveniles and 114,261 lettuce units per year.

The production layouts differ between BFT and CW (Figure 1) mainly in filtration and aeration structures. In the biofloc, two clarifiers in series were designed to receive the water from the fish tank, each with an air lift system adapted in the lower section to direct the flocs to a 6 m^3 mineralizing tank, made of geomembrane, to reduce the accumulation of flakes in the plant's root. It was estimated that the flow rate 1 L min^{-1} in the mineralizer would be sufficient for the water to remain for five days under strong aeration; after this period, the aeration must be turned off for a few minutes so that the supernatant should go to the hydroponic bed and the flocs return to the fish tanks. As for the supernatant water in the decanters, it returns by gravity to the hydroponic bed. The design of the decanters and aeration was calculated according to Hargreaves et al. (2013), whereas in the recirculation system with clear-water the mechanical filters (clarifier and bag), biological and aeration followed the calculations of Lekang (2007) for an aquaculture recirculation system based in the quantities of feed offered. The differences between filter systems resulted in distinct areas and volumes of water in each system. Thus, areas of 598 m^2 (BFT) and 593 m^2 (CW) were required, considering 15 m^2 of supply and office space, 151 m^2 (BFT) and 146 m^2 (CW) for fish production units, including tanks and filtration systems, an area of 432 m^2 for an agricultural greenhouse containing the hydroponic bed. The total volume (fish + plant) in the BFT will be 248.6 m^3 and in the CW at 241.3 m^3.

2.2. *Planning of production*

Fish with 1 g would be stocked and after 46 days harvested with 30 g. The lettuces would be planted with 30 days post sowing and harvested after 23 days of production. The production scheduling required that the fish be harvested at a frequency of every 23 days. Thus, in the first stocking, only half of the productive structure would be used, and then the other half used after 23 days. Consequently, after 23 days of the start of production, only lettuces (half of the structure capacity) would be sold, while on the 46th day juveniles of tilapia (always half of the total capacity) and lettuces (total capacity). The model of harvesting and stocking on the 46th day is repeated throughout the project period. Because of this, the schedule of operating costs and revenues differs between the first and the other evaluated years. In general, the production system was designed to produce approximately 280,000 juveniles of tilapia and 114,261 lettuce units per year, regardless of the technology (BFT or CW) used. The selling price used for 1,000 tilapia juveniles with 30 g was US$ 136.50 and US$ 0.43 per unit of lettuce.

2.3. Operating costs

The amount of feed was calculated considering that the fish would be fed with commercial diets containing 36% crude protein (US$ 0.63 kg), in the average proportion of 10% of live weight. In this manner, feed in the BFT and CW systems, would be approximately 6.8 and 7.1 tons per year, respectively. The molasses powder would be the external source of carbon used to maintain the C: N ratio between 17 and 20:1 in the BFT. The quantities supplied were calculated according to Emerenciano et al. (2017). The total volumes of water for production were considered in the investment, while the annual replenishment of 10% of the total volume in the operational costs. In relation to electrical energy, the main items considered were: submerged pump to recirculate water (140 W in both systems) and air blower for aeration (7.5 kW in BFT and 5.5 kW in CW), besides an estimate of the electricity used by lamps and equipment (e.g. scale, refrigerator, etc.). The difference in air blower between the two systems is related to the superior demand of aeration and water movement in the biofloc system. The land lease fee was considered as operating cost, valued at 10% of revenues (regional measure for rural lease).

To carry out daily production managements, two employees would be required to work eight hours a day, while administrative activities (8 hours per day) would be by the production owner. A monthly base salary of US$ 412.70 per worker plus 43% of social contributions was stipulated. It was considered as the payment of the Special Contribution for Rural Social Security (CESSR) at 2.3% of all revenue collected. The services of daily laborers would be contracted during the periods of plant and fish harvest, stipulated at US$ 3.17 per hour worked. The technical consulting service was included, considering the provision of service for one hour per week at US$ 47.62 per hour.

2.4. Cash flow and economic viability indicators

The project horizon was determined based on the useful life of most of the items considered in the investment (Lapponi, 2000; Engle, 2010). Thus, the investment project was analyzed for a 10-year period and the Annual Interest Rate (AIR) considered was 6% per year. Investments and working capital were included in the outflows at the first year of the cash flow; from the beginning of production, operating costs began to be considered. Working capital was based on the value of operating costs to produce a fish cycle. The inflows considered were revenues, the value of scrap (approximately 10% of the investment items) and at the end of the operation the residual value (Residual value

(US$) = total value (US$) - project horizon (years) x depreciation (US$ / year)) and the recovery of working capital required to start production (Lapponi, 2000; Engle, 2010).

The economic viability indicators were calculated following Faro (1979) and Engle (2010): Internal Rate of Return (IRR); Net Present Value (NPV); PayBack (PB); Discounted PayBack (DPB); Benefit/Cost ratio (B/C) and the Discounted Benefit/Cost ratio (DB/DC). The project was considered viable when IRR = AIR, NPV = 0, DPB < 10 (project period) and DB/DC > 1. In the case where the project has not demonstrated feasibility in any evaluated scenarios, the quantities of plants that should be visually apt for the project to be economically profitable were calculated. The break-even point between revenue and costs would be found to change the economic viability indicators. The altered value to reach the desired equilibrium point was the revenue from lettuces from the second year of the cash flow; the other values were measured based on the proportions and projections found between the years/scenarios.

3. Results

The total investment to start the project using BFT and CW was US$ 55,705 and US$ 56,597, respectively. All investment-related items were summarized and presented in large groups (Table 2). Table 2 also shows the values of depreciation and remnant (scrab) which, together with the useful life of each item and the project duration (10 years), were used to calculate the total residual value. It should be noted that the remnant of items with a useful life of less than 10 years was not considered, since they are disposable and of no value (e.g. bucket, landing-net, expanded clay, etc.). However, it was necessary to reinvest these items in the amount of US$ 172.50 (BFT and CW) every two years, US$ 5,355.34 (BFT and CW) every three years, US$ 1,022.22 (BFT) and US$ 1,065.75 (CW) in the fifth year.

Table 2. Total values (US$) of the initial investment, depreciation, residual and remnant value for implantation of the aquaponic production of Nile tilapia juvenile and lettuce, using biofloc technology (BFT) and clear-water recirculation (CW), to produce approximately 280,000 juveniles and 114,261 lettuce units per year.

Items	**BFT**	**CW**	**% BFT**	**% CW**
Construction general (material + labor)	5,714	5,714	10.1	10.3
Construction fish (material + labor)	5,990	6,958	12.3	10.8
Agricultural Greenhouse (material + labor)	17,143	17,143	30.2	30.8
Production structures (fish)	4,065	4,065	7.2	7.3
Production structures (plant)	9,130	9,130	16.4	16.4
Production structures (filters)	5,763	6,023	10.6	10.3
Aeration	3,382	3,078	5.4	6.1
Equipment and utensils	1,061	1,061	1.9	1.9
Labor installation (aeration and hydraulics)	519	519	0.9	0.9
Office material	381	381	0.7	0.7
Project	1,429	1,429	2.5	2.6
Water	1,128	1,094	1.9	2.0
Total INVESTMENT	55,705	56,597	**100**	**100**
Total DEPRECIATION	5,767	5,843		
Total RESIDUAL	8,264	8,013		
Total SCRAP	4,595	4,712		

* All prices used in the calculation were observed in September 2017 in Sao Paulo State, Brazil (quotation in September: US$ 1 = R$ 3.15).

The operating costs (OC) (Table 3, Figure 2) and total revenues (Table 4) were determined according to the production volume per year, technology and the scenario evaluated, following the production schedule. Figure 2 shows the representation of the general items in the operational costs of each scenario as of year 2. The OC and revenues were on average 2% and 13% lower in year 1 compared to the other years evaluated. The amounts referring to working capital were US$ 7,190 (BFT: 37%), US$ 7,664 (CW: 98%), US$ 7,852 (BFT: 100%) and US$ 7,690 (CW: 100%), considered at the starting point and year 10 of the cash flow.

Table 3. Operating costs (US$) per year. Technology and scenario analyzed for the aquaponic production of Nile tilapia juvenile and lettuce, using biofloc technology (BFT) and clear-water recirculation (CW), to produce approximately 280,000 juveniles and 114,261 lettuce units per year.

Technology: scenario	BFT: 37%		CW: 98%		BFT: 100%		CW: 100%	
Year	1	2 to 10	1	2 to 10	1	2 to 10	1	2 to 10
Feedstock								
Fingerlings	10,971	10,971	10,971	10,971	10,971	10,971	10,971	10,971
Plastic transportation	274	274	274	274	274	274	274	274
Seedlings of lettuce	1,594	1,646	1,594	1,646	1,594	1,646	1,594	1,646
Cone bag (for lettuce)	1,050	1,083	2,764	2,853	2,834	2,926	2,834	2,926
Feed (36% CP - 1.7 mm)	4,318	4,386	4,470	4,541	4,318	4,386	4,470	4,541
Molasses	44	45	0	0	44	45	0	0
Water analysis	750	762	750	762	750	762	750	762
Analysis of specific nutrients	800	813	800	813	800	813	800	813
Fees and taxes								
Land lease	5,019	5,671	7,726	8,658	7,837	8,780	7,837	8,780
CESSR	1,155	1,304	1,777	1,991	1,803	2,019	1,803	2,019
Labor								
Owner	13,694	13,694	13,694	13,694	13,694	13,694	13,694	13,694
Diarist	1,209	1,209	1,209	1,209	1,209	1,209	1,209	1,209
Technical consultancy	1,511	1,511	1,511	1,511	1,511	1,511	1,511	1,511
Worker (general services)	6,847	6,847	6,847	6,847	6,847	6,847	6,847	815
General costs								
Fuel	453	453	453	453	453	453	453	453
Maintenance / repairs	2,015	2,015	2,015	2,015	2,015	2,015	2,015	2,015
Electricity	5,312	5,312	3,921	3,921	5,312	5,312	3,921	3,921
Water replenishment	37	37	36	36	37	37	36	36
Total	57,054	58,035	60,815	62,197	62,304	63,701	61,020	62,419

* All prices used in the calculation were observed in September 2017 in Sao Paulo State, Brazil (quotation in September: US$ 1 = R$ 3.15).

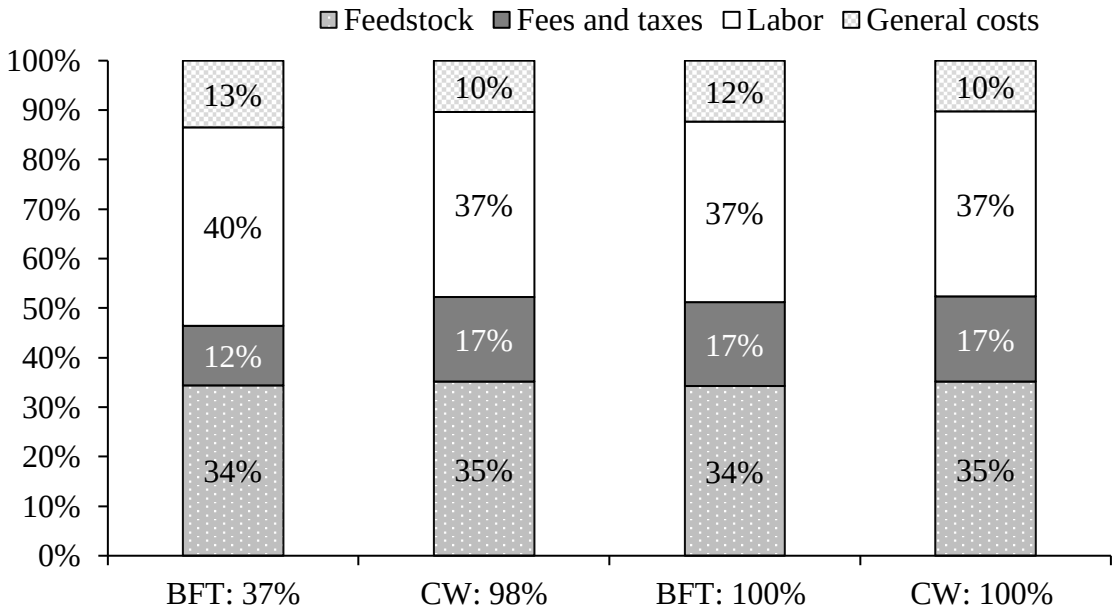


Figure 2. Representative (%) of the general items in operating costs from year 2. Technology and scenario analyzed for the aquaponic production of Nile tilapia juvenile and lettuce, using biofloc technology (BFT) and clear-water recirculation (CW), to produce approximately 280,000 juveniles and 114,261 lettuce units per year.

Table 4. Revenues (US$) per year. Technology and scenario analyzed for the aquaponic production of Nile tilapia juvenile and lettuce, using biofloc technology (BFT) and clear-water recirculation (CW), to produce approximately 280,000 juveniles and 114,261 lettuce units per year.

Technology: scenario	Year	Lettuce	Tilapia	Total	% Lettuce	% Tilapia
BFT: 37%	1	16,571	33,623	50,194	33	67
	2 to 10	18,286	38,426	56,711	32	68
CW: 98%	1	43,638	33,623	77,261	56	44
	2 to 10	48,152	38,426	86,578	56	44
BFT: 100%	1	44,743	33,623	78,365	57	43
	2 to 10	49,371	38,426	87,797	56	44
CW: 100%	1	44,743	33,623	78,365	57	43
	2 to 10	49,371	38,426	87,797	56	44

* All prices used in the calculation were observed in September 2017 in Sao Paulo State, Brazil (quotation in September: US$ 1 = R$ 3.15).

The inputs and outputs were organized in the cash flow, making it possible to calculate the economic feasibility indicators within the four proposed scenarios (Table 5). The projects for BFT: 100% and CW: 98% and 100% were economically viable. However, lettuce production integrated with Nile tilapia juveniles based on the productive results of plants in biofloc obtained experimentally (Article 1) was not feasible (excess of C and D grades). In this way, it was calculated how much of plants A and B would need to be produced for the project to be viable, having as parameters TIR=6% a and NPV=0. It was calculated that 63.46% of plant production should have desirable visual characteristics for marketing.

Table 5. Economic indicators of the different scenarios for the aquaponic production of Nile tilapia juvenile and lettuce, using biofloc technology (BFT) and clear-water recirculation (CW), to produce approximately 280,000 juveniles and 114,261 lettuce units per year.

Economic indicators	BFT: 37%	AC: 98%	BFT: 100%	AC: 100%
IRR (%)	- 21.14	31.42	30.84	32.20
NPV (US$)	-79,513	107,097	104,932	108,929
PayBack (years)	No Payback	4.16	4.21	4.07
Discounted PayBack (years)	No Payback	4.55	4.61	4.44
Benefit/Cost ratio	0.88	1.25	1.24	1.25
Discounted Benefit/Cost ratio	0.84	1.20	1.19	1.20

4. Discussion

The investment to start the project was similar among the technologies evaluated. A larger difference was expected than the calculated US$ 892, since in cultivation using biofloc technology there is low dependence on filters (Crab et al., 2012) and usually only clarifiers are used for the solids control, coupled to the fish structures of cultivation. However, in aquaponics the maximum balance between the favorable conditions for fish and plants (Yildiz et al., 2017) is desirable, therefore, the need to reduce the particles in the roots of the plants. The investment values showed that the proposed system to reduce the accumulation of flocs in the hydroponic bed (clarifiers and mineralizer) was costly, resulting in a smaller difference between the values for BFT and CW. In Article 1 it was suggested that research aimed at the engineering of the system be carried out to make the

integration between biofloc technology and aquaponics technically feasible. The results of the present study emphasize that these improvements in the system are necessary, nevertheless one must look for alternatives that help to reduce the investment of the project.

The most representative investment items in both technologies are related specifically to plants, especially the greenhouse and productive structures, such as hydroponic bed. These two structures together account for approximately 47% of the total investment, while the construction of the shed and the productive structures of the fish, mainly the tanks, correspond to approximately 19.5%. The greatest investment related to the production of plants is usually reported in aquaponics. Bosma et al. (2017) evaluated the production of catfish integrated to the lettuce and tomato crop and reported a high cost of the greenhouse and the hydroponic bed, reaching 75% of the initial investment. One of the factors that influence this difference between the highest representation of hydroponic structures in investment is the relation between the volume of fish by the plant area (m^3:m^2), which in turn is directly related to the amount of feed (nutrient) required (g) per plant (leafy vegetables) per day. Tokunaga et al. (2015) reported that 80% of the initial investment was allocated to the installation of the hydroponic bed, in tilapia and lettuce aquaponic production using a floating system in Honolulu, Hawaii, USA. The authors used a ratio of 15 m^3 (volume of fish) by 1 m^2 (plant area), that is almost five times greater than that of the present study. The possibility of increasing this proportion, and consequently the volume of production and revenues, can be used as a strategy to expand the aquaponic system in stages. As this is not yet a very dominant technology, starting with small modules and increasing production after gaining experience and consumer market can make integration attractive to the investor.

The operating costs change according to the different production volumes. This is due to the fees (land lease and CESSR) and plastic cones for marketing that are directly related to the revenues and the amount of lettuces offered to the market. The operating costs were also distinct among the technologies, even considering the market characteristics of all lettuces produced. This is because, although in the biofloc system the feed conversion of the fish was lower, the expenses with electric energy surpassed the economy with feed. Rego et al. (2017) evaluated the viability of a shrimp production in a biofloc system compared to a conventional culture, and also found a trend of higher

operational costs in the BFT in compare to the control. They attributed this to the greater use of input (feedstock) by productive area and demand for more skilled labor.

In conventional aquaculture systems, feed is the item that most influences OC, reaching 60% of the total (Scorvo-Filho et al., 2010; Ayroza et al., 2011). However, in aquaponic culture labor is usually reported as the costliest item of expenditure. The results presented in this study corroborates the results by Kodama (2011), Tokunaga et al. (2015) and Bosma et al. (2017), in which approximately 38% of the OC was represented by labor. Quagrainie et al. (2017) reported that labor represented more than 60% of the OC regardless of the volume of production, in an analysis of aquaponic farms with different investments and sizes in the U.S. Midwest. Possibly this higher proportion is related to the constant need of monitoring the biotic and abiotic variables that involve the production and with specialized professionals, that can operate the integrated system. In addition, fluctuations in operating costs may vary with volume, sizing and according to the region where it is being produced; for example, different rates of electric power or frequency of feed availability may alter these ratios (Goddek et al., 2015).

In the revenues, it was seen that in the BFT scenario with 63.46% of the marketable plants (calculated by the break-even point) the lettuces contributed 45% of the total, in the BFT: 37% represented 33% and in all other scenarios 56% of the total. Different authors report that in aquaponic productions in Chile (Emerenciano et al., 2015) and in the United States (Tokunaga et al., 2015) the plants provide the highest financial return, of 70 and 80%, respectively. In addition to the differences in design, characteristics for each situation, this lower relation between the two products revenues (fish and lettuce) is certainly related to the short cycle and the fast cash return of the tilapia juveniles, compared to the growing phase. In this sense, producing juveniles of tilapia in an aquaponic system makes possible the fish being not only the byproduct.

The results of the different economic indicators showed that it is economically feasible to produce tilapia juvenile and butter lettuce in an aquaponic system using clear-water or biofloc technology, yet in the latter the minimum of 63.46% of the plants produced should be visually suitable for the market (Table 5). Except for the BFT: 37%, in all other scenarios NPV were above 0, revealing that the investment will be recovered, remunerated at a rate of 6% per year. The values of DPB revealed the year in which the initial investment and accumulated net income equals, indicating that in 4.55 (CW: 98%), 4.61 (BFT: 100%) and 4.44 (CW: 100%) years the project will be paid. Meanwhile the

calculated IRR showed at what rate the project will have NPV=0, that is, at 31.42 (CW: 98%), 30.84 (BFT: 100%) and 32.20% (CW: 100%). Lastly, the DB/DC ratio above 1.00 also indicated projects that are economically viable.

Some handling or minor changes to the project could make it even more profitable. As for example, growing different plants in a single system, providing greater nutrient utilization and diversification of production, thus reducing dependence on market fluctuations of a single product (Bailey and Ferrarezi, 2017). Capturing consumers who recognize aggregate ecological value and are willing to pay more for it can increase production revenues (Goddek et al., 2015; Pinho et al., 2017b). Another important factor to consider is to include aquaponic production on an existing aquaculture farm. The production of its own juvenile can help to reduce operating costs and segment production efficiently, causing positive effects on growing phase. In addition, there is the extra revenue from the marketing of differentiated vegetables.

Among all the scenarios evaluated, the CW presented the best results. In this sense, it is economically more advantageous to invest in structures for aquaponic production using CW than BFT, even if the latter reaches 100% of the plant's production. However, investing in research that develops mechanisms that enable better plant performance may be useful for BFT tilapia producers. That is because, in addition to providing diversification in production and increasing income, integration with hydroponics can increase the sustainability of production and make it more resilient.

5. Conclusion

It is concluded that it is economically feasible to produce tilapia juveniles and butter lettuce in an aquaponic system, using clear-water recirculation systems or adopting biofloc technology, provided that at least 63.46% of the plants produced are visually suitable for commercialization. Among the technologies evaluated with 100% of the plant's production, the CW system is shown as the mostprofitable.

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7. CONSIDERAÇÕES FINAIS

Nas condições do presente estudo, conclui-se que a aplicação da tecnologia de bioflocos na produção aquapônica possibilita melhora dos parâmetros zootécnicos dos juvenis de tilápia-do-nilo. No entanto, quando maduro, prejudica o desempenho fitotécnico e as características visuais das alfaces. Além disso, os peixes cultivados nos sistemas de bioflocos e água clara apresentam semelhanças nas curvas de crescimento, condições nutricionais (composição centesimal e de ácidos graxos na carcaça) e nos padrões morfológico e morfométrico das fibras musculares. Quanto à análise de viabilidade econômica, esta indica que é viável a produção utilizando CW ou BFT, desde que, neste último, o mínimo de 63,5% das plantas produzidas seja visualmente aptas para comercialização. Desta maneira, a integração só será tecnicamente e economicamente viável se mecanismos e alterações na engenharia do sistema forem desenvolvidos para melhorar o desempenho das plantas e a disponibilidade de alimento natural para os peixes.

Recomendações para futuras investigações:

- Testar a frequência e os mecanismos de utilização do sedimentador em sistemas de bioflocos na aquaponia, com ênfase na diminuição dos sólidos sedimentáveis (bioflocos) na bancada hidropônica;
- Testar o emprego da tecnologia de bioflocos em aquapônia com adoção dos sistemas desacoplados ou DAPS (do inglês "*Decoupled Aquaponic Systems*");
- Realizar estudos mais avançados sobre a relação dos microrganismos dos bioflocos com a biodisponibilidade dos nutrientes para as plantas;
- Aplicar métodos e ferramentas para mensurar o real caráter sustentável da integração da tecnologia de bioflocos na aquaponia.

8. REFERÊNCIAS INTRODUÇÃO GERAL

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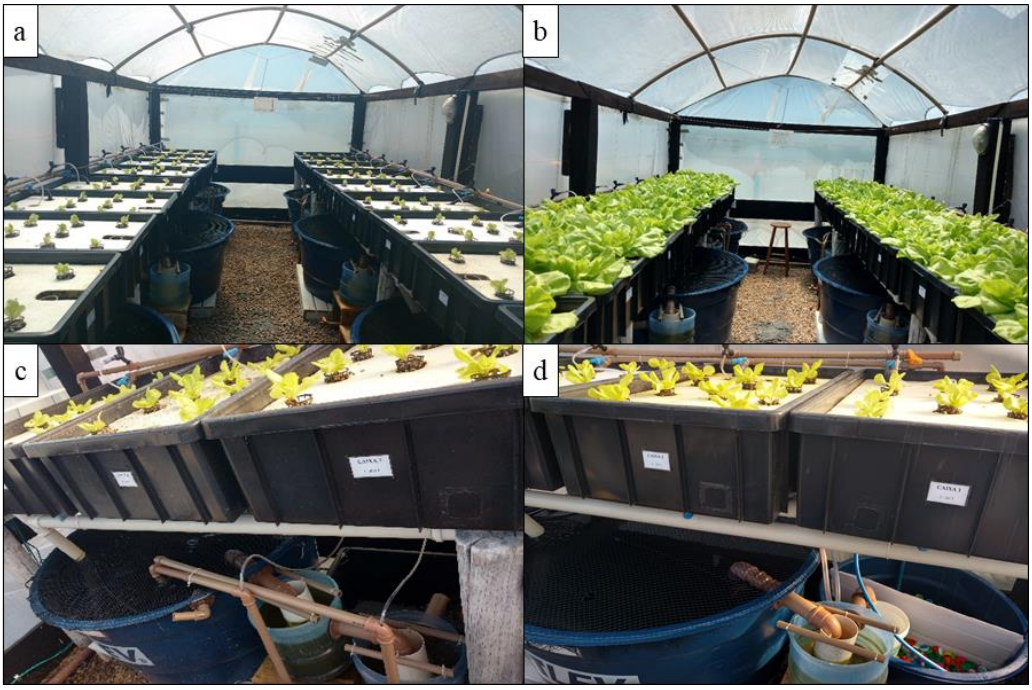
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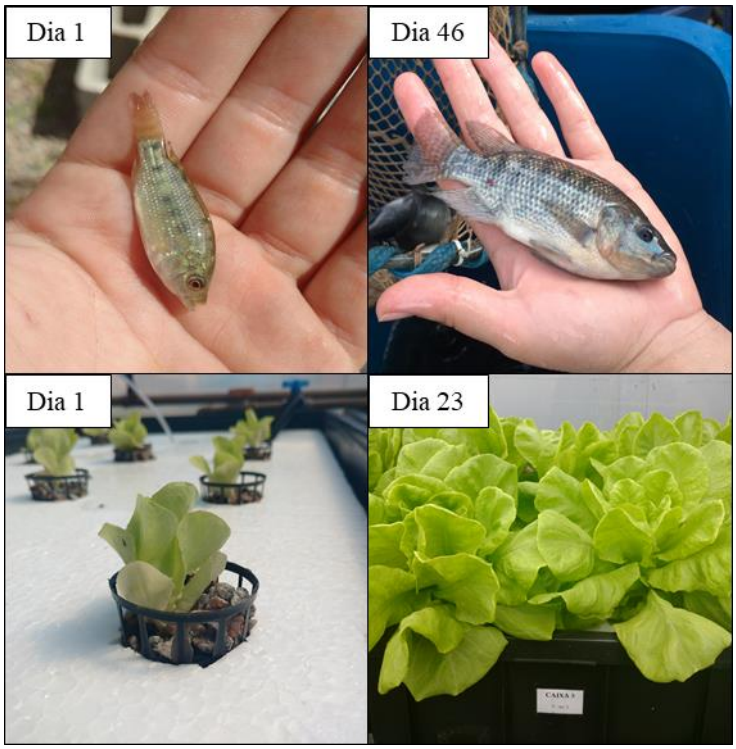
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9. ANEXOS

Anexo 1. Fotos do dispositivo utilizado no experimento. a – visão geral no 1º dia do ciclo 1, b – visão geral no 22º dia do ciclo 1, c – sistema de bioflocos, d – sistema de água clara.



Anexo 2. Fotos ilustrando o desenvolvimento dos peixes e plantas no sistema aquapônico.



Anexo 3. Volume do floco (mL L^{-1}) no tanque das plantas e dos peixes no final do período experimental (46 dias).

