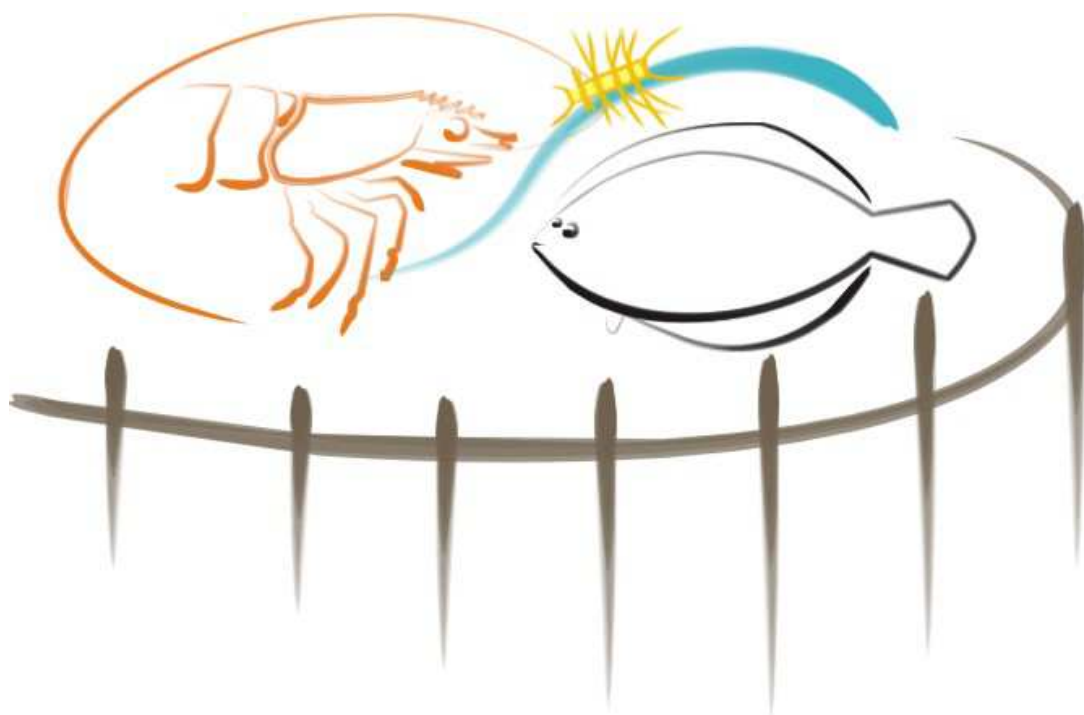


UNIVERSIDADE FEDERAL DO RIO GRANDE – FURG
INSTITUTO DE OCEANOGRAFIA
PROGRAMA DE PÓS GRADUAÇÃO EM AQUICULTURA



**CONTROLE DE pH E DUREZA TOTAL EM SISTEMA DE BIOFLOCOS,
AVALIANDO A QUALIDADE DA ÁGUA E O DESEMPENHO DE TILÁPIA
DO NILO *Oreochromis niloticus* (L.)**

GABRIEL BERNARDES MARTINS

FURG
Rio Grande, RS
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Tese apresentada como parte dos requisitos para a obtenção do grau de doutor em Aquicultura no programa de Pós-Graduação em Aquicultura da Universidade Federal do Rio Grande.

Orientador: Dr. Ricardo Berteaux Robaldo

FURG
Rio Grande, RS
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RESUMO GERAL

Em sistema de bioflocos (BFT), a alta biomassa de peixes e matéria orgânica, associadas à ciclagem dos nitrogenados inorgânicos, ocasionam intenso consumo da alcalinidade e redução do pH. Portanto, são necessárias frequentes adições de composto alcalinizante. Nesse contexto, como forma de aprimorar o sistema BFT para tilápias do Nilo *Oreochromis niloticus*, este estudo avalia a utilização de fontes alcalinizantes e valores de pH e dureza, verificando o desempenho de crescimento e a qualidade da água durante a fase de berçário. Todos os ensaios foram realizados no “Laboratório de Piscicultura do Chasqueiro”, da Universidade Federal de Pelotas. Para os ensaios, foram utilizadas caixas com 37.5 L de volume útil, aeração contínua (pedra porosa), aquecedor (28°C) e três réplicas para cada tratamento. O primeiro capítulo, realizado durante 60 dias, demonstra que o melhor desempenho de crescimento e produtividade ocorre ao utilizar bicarbonato de sódio (NaHCO_3) ($44,1 \pm 0,9$ g e $23,5 \pm 0,5$ kg/m³), comparado ao carbonato de cálcio (CaCO_3) ($38,3 \pm 1,3$ g e $20,8 \pm 0,70$ kg/m³), enquanto o hidróxido de cálcio (Ca(OH)_2) ($40,6 \pm 1,7$ g e $21,6 \pm 0,9$ kg/m³) foi similar aos demais. O crescimento reduzido para o tratamento CaCO_3 foi atribuído ao excessivo aumento dos sólidos suspensos totais (SST) e maior frequência de clarificações, que conseqüentemente reduzem o teor de proteínas totais dos bioflocos. Além disso, ao utilizar Ca(OH)_2 ou CaCO_3 , a água tornou-se extremamente dura (>1000 mg/L CaCO_3), entretanto não parece ter comprometido o crescimento. A sobrevivência (aprox. 80%) e a conversão alimentar (aprox. 1,1) não diferiram entre os tratamentos. Para avaliar o status fisiológico dos peixes, ao final do ensaio foram coletados tecidos (sangue, fígado e brânquias) para avaliação de parâmetros hematológicos e da capacidade antioxidante. Ambos os parâmetros não demonstraram alteração, indicando que a utilização dos alcalinizantes não provoca distúrbio fisiológico. O segundo capítulo, testando os de pH 6,5, 7,5 e 8,3 durante 60 dias, demonstrou crescimento e produtividade superiores para o pH 7,5 ($44,1 \pm 0,9$ g e $23,5 \pm 0,5$ kg/m³), comparado ao 8,3 ($37,1 \pm 3,9$ g e $19,1 \pm 2,0$ kg/m³), enquanto o 6,5 ($40,4 \pm 4,1$ g e $22,6 \pm 2,3$ kg/m³) foi similar aos demais. A sobrevivência (aprox. 80%) e a conversão alimentar (aprox. 1,1) não diferiram entre os tratamentos. Os resultados indicam ser vantajoso à nitrificação iniciar a formação dos bioflocos com pH próximo a 8,0, e que a manutenção até pH 6,5 não compromete a

ciclagem dos nitrogenados. Ainda mais, a manutenção do sistema com pH mais baixos reduz a produção de sólidos, o que favorece o aumento do teor protéico dos bioflocos, devido a menor necessidade de remover sólidos. As avaliações hematológicas e oxidativas não demonstraram diferença entre os tratamentos, sugerindo que não é um desafio às tilápias o pH entre 6,5 e 8,3. No terceiro capítulo, ao testar as concentrações de dureza (250,0, 700,0 mg/L CaCO₃ e controle (79,0 mg/L CaCO₃) por 20 dias, utilizando CaCl₂, o melhor desempenho de crescimento e produtividade ocorreu para 250 (7,6 ± 0,4 g e 4,8 ± 0,2 kg/m³) e 700 (7,4 ± 0,3 g e 4,6 ± 0,05 kg/m³), comparado ao controle (6,3 ± 0,1 g e 3,6 ± 0,4 kg/m³). Enquanto a sobrevivência (aprox. 80%) e a taxa de conversão alimentar (aprox. 1,0), não diferiram entre os tratamentos. As concentrações de SST demonstrou elevação positiva com o aumento da dureza. Isso deve-se à maior adesão de Ca⁺² na estrutura dos bioflocos, aumentando a densidade, o que torna o índice de volume de floco (FVI, mL/g) nos tratamentos com correção da dureza mais estável ao longo do ensaio. Finalmente, como forma de otimizar o desempenho, os resultados obtidos podem ser utilizados na produção de tilápias do Nilo *O. niloticus* em sistema BFT, indicando a formação de bioflocos usando pH em torno de 8,0, manutenção em pH até 6,5, como também a utilização dos alcalinizantes NaHCO₃ ou Ca(OH)₂. Ainda mais, a utilização de Ca(OH)₂ causando elevação excessiva da dureza, não compromete o crescimento quando mantida até 700 mg/L CaCO₃.

Palavras chave: berçário, superintensivo, íons, sólidos suspensos totais, densidade biofloco

ABSTRACT

In biofloc technology (BFT), the high biomass of fishes and organic material, associated cycling of inorganic nitrogenous, cause intense alkalinity consumption and pH reduction. Thus, are necessary add frequently alkalizing compound. In this context, as a way to improve the system for Nile tilapia *Oreochromis niloticus*, this study evaluate the use of alkalizing compounds, pH and total hardness levels, verifying the growth performance and water quality. All assays were performed in “*Laboratório de Piscicultura do Chasqueiro*”, of the *Universidade Federal de Pelotas*, Rio Grande do

Sul, BR. For the experiments, were utilize tanks (useful vol. 37.5 L) and continuous aeration (two airstone), heat (100W, adjusted to 28°C) and three replicates for treatment. The first chapter realized for 60 days, demonstrate that best growth and net yield performance occur to utilize NaHCO_3 (44.1 ± 0.9 g and 23.5 ± 0.5 kg/m³), compared to CaCO_3 (38.3 ± 1.3 g and 20.8 ± 0.70 kg/m³), while the Ca(OH)_2 (40.6 ± 1.7 g and 21.6 ± 0.9 kg/m³) was similar to the other treatments. The reduced growth for CaCO_3 was attributed to excessive total solid suspension (TSS) increase and higher clarifications frequency that consequently reduces the protein content of bioflocs. Besides that, to utilize Ca(OH)_2 or CaCO_3 , the water became very hard (>1000 mg/L), however, no appear to have compromised growth. The survival (about 80%) and food conversion rate (FCR) (about 1.1) were similar among treatments. For evaluate the physiologic status of fishes, at final assay were collected tissue (blood, liver and gills) for evaluate hematologic parameters and antioxidant capacity. Both parameters do not demonstrate alteration, indicating that use of alkalizing do not cause physiologic disturb. The second chapter, testing the pH levels at 6.5, 7.5 and 8.3 during 60 days, demonstrate superior growth and net yield for pH 7.5 (44.1 ± 0.9 g and 23.5 ± 0.5 kg/m³), compared to 8.3 (37.1 ± 3.9 g and 19.1 ± 2.0 kg/m³), while the 6.5 (40.4 ± 4.1 g and 22.6 ± 2.3 kg/m³) was similar to other treatments. The survival (about 80%) and FCR (about 1.1) was equal among treatments. The results indicate be benefic to nitrification initiate biofloc formation with pH around the 8.0, and that maintenance at pH 6.5 do not compromises the nitrogenous cycling. Furthermore, the system maintenance with low pH reduces the solid suspension production, which favor the crude protein increase in bioflocs, because the less need to clarifications. The hematologic and oxidative evaluations, do not demonstrate difference among treatments, suggesting that it is not challenge to Nile tilapia the pH between 6.5–8.3. On third chapter, to test total hardness levels (250, 700 mg/L CaCO_3 and control) at 20 days, utilizing CaCl_2 , the best growth and net yield performance occur for 250 (7.6 ± 0.4 g and 4.8 ± 0.2 kg/m³) e 700 (7.4 ± 0.3 g and 4.6 ± 0.05 kg/m³), compared to control (6.3 ± 0.1 g and 3.6 ± 0.4 kg/m³). While the survival (about 80%) and FCR (about 1.0) do not differ among treatments. Interestingly, the TSS levels demonstrate positive relation with increase of total hardness. This is due to the calcium adhesion in biofloc structure, increasing the density, what makes the floc volume index (FVI) in treatments

more stable throughout assay. Finally, as a way to improve the performance, the results obtained may be used on Nile tilapia production in BFT system, indicating the biofloc formation using pH around of the 8.0 and maintenance until 6.5, as also NaHCO_3 or Ca(OH)_2 such alkalizing. Still more, the Ca(OH)_2 utilization that causes excessive increased in the total hardness, do not compromises the growth until 700 mg/L CaCO_3 .
Keywords: superintensive nursery, ions, total solid suspension, biofloc density.

INTRODUÇÃO GERAL

O desenvolvimento da aquicultura tem enfrentado desafios de sustentabilidade, relacionados ao consumo da água e produção de efluentes, uso de óleo e farinha de peixe nas rações, utilização de espécies exóticas e também perdas por doenças na produção (Colt 2006, Naylor 2000, Deines et al. 2016, Defoirdt et al. 2010). Nesse contexto, as pesquisas desenvolvidas para tilápia do Nilo *Oreochromis niloticus* em tecnologia dos bioflocos (BFT) têm demonstrado bons resultados, sendo considerado um sistema que supre grande parte das variáveis relacionadas à sustentabilidade (De Schryver et al. 2008, Crab et al. 2012).

O sistema BFT, é baseado na ciclagem do nitrogênio (N) inorgânico, realizada por microorganismos, que aderidos formam os bioflocos. Esses são compostos principalmente por bactérias, fungos, microalgas, zooplâncton, como também colóides, polímeros orgânicos, cátions e matéria orgânica (De Schryver et al. 2008). Além disso, os bioflocos possuem como características o formato irregular, tamanhos diversos, alta porosidade e permeabilidade aos fluidos (Chu & Lee 2004, Crab et al. 2012).

A ciclagem do N inorgânico, pelos microorganismos, permite que as renovações de água sejam mínimas. Para a produção de peixes em sistema BFT, o consumo é menor que 200 L de água/kg peixe produzido, enquanto que em viveiros convencionais, utiliza aprox. 20000 L/kg (Martínez-Córdova et al. 2016). Assim, a baixa renovação de água permite que ocorra a ciclagem dos restos de ração, matéria orgânica e N inorgânico, pelos microorganismos em sistema BFT.

Quando em suspensão na água, os bioflocos são consumidos pelos peixes, portanto, são fontes extra de nutrientes (De Schryver et al. 2008). Tem sido demonstrado que o conteúdo de proteína, lipídeos e carboidratos (% base seca) dos

bioflocos estão no intervalo entre 12-42%, 0,5-12% e 18-36%, respectivamente (Avnimelech 2012). Embora a concentração lipídica seja tipicamente baixa, os bioflocos são considerados importantes fontes de ácidos graxos essenciais (n-3 e n-6) (Azim & Little 2008, Ekasari et al. 2010), que são encontrados particularmente em óleo e farinha de peixe, e não compõe os substitutos vegetais das rações (Martínez-Córdova et al. 2016).

Trabalhos prévios foram realizados abordando o maior aproveitamento dos nutrientes por tilápias em sistema BFT, e demonstraram maior assimilação de nitrogênio em relação a sistemas convencionais (43 vs 23%, respectivamente) (Avnimelech 2006), consequentemente reduzindo o consumo de ração entre 20-30% (Avnimelech et al. 1994, Avnimelech 2007, Avnimelech & Kochba 2009, Ekasari et al. 2014). Nesse contexto, a baixa utilização de água e o melhor aproveitamento dos nutrientes comprovam a sustentabilidade do sistema de bioflocos.

O sistema BFT foi inicialmente desenvolvido realizando fertilizações com carbono (C) orgânico, mantendo a relação entre C orgânico e nitrogênio (N) entre 15-20:1, para estimular o crescimento de bactérias heterotróficas (equação 1) (Avnimelech 1999). Esse grupo possui vantagens importantes, como o rápido crescimento e a grande capacidade de absorver o nitrogênio amoniacal.



Diferentes estratégias de fertilização orgânica têm sido propostas para a formação dos bioflocos na produção de tilápias do Nilo. Como por ex., a utilização de amido (Crab et al. 2009), glicerol, glicose (Ekasari et al. 2010), melão ou bagaço de cana de açúcar (Hargreaves 2013). Além da fonte utilizada, a manutenção da relação C:N é fundamental, e recentemente Pérez-Fuentes et al. (2016) demonstraram melhor desempenho de crescimento e produtividade de tilápias do Nilo ao manter a relação 10:1, comparado a 12,5 até 20:1.

Devido ao consumo de C orgânico (Tabela 1) e rápido crescimento, as bactérias heterotróficas produzem grande quantidade de biomassa, elevando rapidamente a quantidade de sólidos no sistema, com elevado consumo de O₂ e produção de CO₂, que favorece a acidificação do meio. Além disso, tem sido relatada em sistema BFT,

utilizando essencialmente bactérias heterotróficas, a ocorrência de oscilações na capacidade de absorver N, causando aumentos abruptos de amônia (Azim & Little 2008).

Tabela 1. Balanço estequiométrico para a metabolização de 1,0 g de NH_4^+ por bactérias heterotróficas e autotróficas.

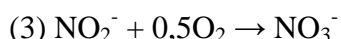
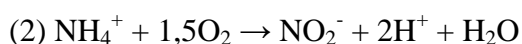
Consome	Heterotrófica	Autotrófica
NH_4^+ (g)	1,0	1,0
C orgânico (g C/g N)	15,17	-
Alcalinidade (g Alc./g N)	3,57	7,05
Oxigênio dissolvido (g O_2 /g N)	4,71	4,18
Produz		
Sólidos (g Sól./g N)	8,07	0,2
NO_3^- (g NO_3^- /g N)	-	0,98
CO_2 (g CO_2 /g N)	9,65	5,85

Adaptado de Ebeling et al. (2006).

Como forma de minimizar essas dificuldades, estudos posteriores demonstram a possibilidade de manter, além das bactérias heterotróficas, o grupo quimioautotrófico (autotrófico) associado aos bioflocos (Avnimelech 2012, Ray & Lotz 2014). Isso ocorre ao condicionar a uma baixa relação C:N no sistema, seja pelas estratégias de fertilização com C orgânico ou apenas pela ração.

As autotróficas são responsáveis pela nitrificação, que ocorre em duas etapas: primeiramente a amônia é oxidada à nitrito (equação 2), e posteriormente à nitrato (equação 3). A primeira etapa é realizada pelo grupo denominado Bactérias Oxidantes de Amônia (AOB) e a esse grupo pertencem os gêneros: *Nitrosococcus*, *Nitrosospira* e *Nitrossomonas* (Koops & Pommerening-Röser 2001, Schreier et al. 2010). Enquanto a

segunda etapa, a oxidação de nitrito à nitrato, é realizada pelo grupo denominado Bactérias Oxidantes de Nitrito (NOB), e inclui os gêneros: *Nitrobacter* e *Nitrospira* (Brown et al. 2013, Rurangwa & Verdegem 2015).

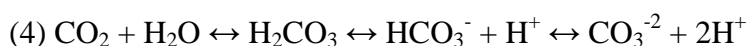


A taxa de nitrificação é influenciada principalmente pela concentração de substrato (amônia ou nitrito) e parâmetros de qualidade da água, como: temperatura, pH, alcalinidade e salinidade (Chen et al. 2006, Gujer 2010, Gonzalez-Silva et al. 2016). A nitrificação possui grande influência na redução do pH, especialmente devido a produção de H^+ , e grande consumo de carbono inorgânico proveniente da alcalinidade. E por sua vez, o pH afeta a nitrificação, devido a ativação-desativação das bactérias nitrificantes, efeitos nutricionais relacionados a alcalinidade (carbono inorgânico) e inibição das bactérias por amônia livre e ácido nitroso livre ($\text{NH}_3/\text{NH}_4^+$ e $\text{NO}_2^-/\text{HNO}_2$) (Villaverde et al. 1997, Bock & Wagner 2001, Gieseke et al. 2006).

Estudos em sistemas de tratamentos de efluentes foram realizados para demonstrar a influência do pH na taxa de nitrificação. Como por ex. Jimenez et al. (2011), que ao testar pH 6,5 não detectaram atividade nitrificante em lodo ativado, entretanto, para o intervalo entre 7,5–9,9 não foi detectada inibição. Enquanto Gujer (2010), cita em sua revisão, o intervalo ideal para a nitrificação entre 6,2–8,0. Diferentemente, as bactérias heterotróficas são menos influenciadas pelo pH, podendo ser considerado o intervalo ideal entre 7,0 – 8,0, porém até 10,0 é relatado crescimento (Mayo & Noike 1996).

O consumo da alcalinidade e do pH tem sido previamente relatado em sistema BFT, devido à ciclagem do N inorgânico (absorção pelas heterotróficas e nitrificação), como também pela oxidação da matéria orgânica e produção de CO_2 pelos organismos no sistema (Azim & Little 2008; Pérez-Fuentes et al. 2016). Pode ser considerado um desafio a preservação de valores adequados de pH e alcalinidade em sistema BFT, que sejam capazes de manter a máxima absorção dos nitrogenados inorgânicos.

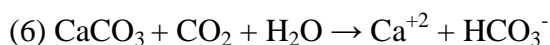
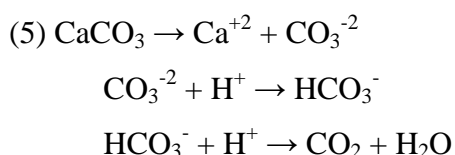
Portanto, a utilização de compostos alcalinizantes é fundamental para a manutenção do sistema tampão da água (equação 4). A alcalinidade total, que é associada ao tamponamento da água, é representada pela soma das bases tituláveis, que são: HCO_3^- , CO_3^{2-} e OH^- , e é expressa em mg/L CaCO_3 (Boyd et al. 2016).



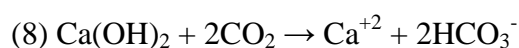
Em aquicultura, é recomendada a manutenção da alcalinidade a uma taxa suficiente para manter o pH estável ao longo do dia, e de forma geral, valores entre 50-100 mg/L CaCO_3 são mantidos em sistemas dulceaquícolas (Boyd et al. 2011, Boyd et al. 2016). Assim, são utilizados compostos alcalinizantes, como o bicarbonato de sódio (NaHCO_3), o carbonato de cálcio (CaCO_3) e o hidróxido de cálcio ($\text{Ca}(\text{OH})_2$).

O NaHCO_3 possui rápida diluição na água, e ao se dissociar produz HCO_3^- e Na^+ . É um composto seguro e eficaz no aumento da alcalinidade, e tem sido comumente utilizado em sistemas de recirculação (RAS) (Loyless & Malone 1997). Estudos anteriores com camarão marinho *Litopenaeus vannamei* em BFT demonstraram a eficácia do NaHCO_3 na correção da alcalinidade e do pH (Furtado et al. 2011, Zhang et al. 2015).

O calcário é composto por CaCO_3 e MgCO_3 , e em sistema intensivo tem sido utilizado apenas o carbonato de cálcio (CaCO_3), devido ao maior grau de pureza. Comparado ao NaHCO_3 , o CaCO_3 possui menor capacidade de diluição, sendo menos eficiente na correção da alcalinidade e do pH. Quando dissolvido em água com pH neutro ou superior, a capacidade de dissociação é baixa (equação 5), entretanto, em pH abaixo de 7.0 e na presença de CO_2 , a diluição é facilitada (equação 6) (Boyd et al. 2016).



Enquanto o hidróxido de cálcio, também denominado cal hidratada (Ca(OH)_2), é amplamente utilizado em viveiros escavados, principalmente por ser uma alternativa de baixo custo. Além disso, também tem sido utilizado para a produção de camarão marinho *L. vannamei* em sistema BFT, demonstrando bons resultados (Furtado et al. 2014). O Ca(OH)_2 possui rápida diluição, e devido à abrupta elevação do pH é considerado bactericida (Ganguly et al. 1999). Portanto, sua utilização deve ser realizada com cuidado, especialmente quando em água doce com alcalinidade baixa. A dissolução na água produz as reações das equações 7 e 8.



Devido à baixa ou nula renovação de água, o composto alcalinizante utilizado será responsável pelo acúmulo de íons ao longo do cultivo (Na^+ e/ou Ca^{+2}). Por exemplo, a utilização excessiva de NaHCO_3 por um longo período poderá tornar a água oligohalina ou até salobra. É demonstrado que produzir tilápia do Nilo em água salobra (4-6‰) proporciona melhora no crescimento (Qiang et al. 2013). Entretanto, o aumento somente de Na^+ pode causar desequilíbrio iônico, causando maior gasto energético com a osmorregulação, e em situações severas pode prejudicar o crescimento (Boeuf & Payan 2001). Esse é um tema que não é explorado para tilápias do Nilo, e pode se tornar um limitante para o reuso da água ou descarte como efluente.

A dureza total é representada pela soma dos cátions divalentes, principalmente Ca^{+2} e Mg^{+2} , e é expressa como mg/L de CaCO_3 (Boyd et al. 2016). De acordo com o nível de dureza total, as águas podem ser classificadas como: leve (<50), moderadamente dura (50-150), dura (150-300) e muito dura (>300) (Boyd 2015). Além disso, a partir da dureza total, é possível demonstrar a dureza de Ca^{+2} , que representa 0,4 do valor de dureza total (Boyd et al. 2016).

Os sistemas dulceaquícolas são bastante heterogêneos em relação à dureza, podendo possuir valor entre 5-75 mg/L em regiões úmidas, até 1000 mg/L em áreas com solo calcário (Boyd 2015). Portanto, não há um intervalo de dureza comum à água

doce. Para efeito de comparação, as águas marinhas possuem dureza em torno de 6.300 mg/L CaCO₃, e concentração de Ca⁺² de 400 mg/L (Boyd 2015).

Para os teleósteos, a principal função do Ca⁺² está relacionada ao aumento da rigidez das *tight junctions* nas brânquias, o que evita a perda iônica (Wood 2001). Essa é uma das estratégias também utilizadas para evitar a perda iônica em água com pH acidificado (Baldisserotto 2011). Para tilápias do Nilo, Cavalcante et al. (2012) demonstraram melhor desempenho quando a dureza é mantida superior a 20 mg/L CaCO₃, enquanto que Cavalcante et al. (2014) ao balancear a relação dureza:alcalinidade, demonstraram melhor desempenho quando mantida proporção em 1,0, comparado a 0,5 ou 5,0.

Em outra perspectiva, a função do Ca⁺² é bastante explorada para sistema de tratamento de efluentes que utilizam lodo ativado (similar aos bioflocos utilizados em aquicultura) sendo principalmente relacionado à coagulação-floculação e aumento da força de adesão dos flocos. O Ca⁺² é responsável pela neutralização das cargas negativas dos microorganismos e substâncias poliméricas extracelulares (EPS) produzida pelas bactérias, portanto, promove a adesão dessas estruturas (Peeters et al. 2011, Ye et al. 2016).

Além do aumento da concentração iônica devido à baixa renovação, a utilização da nitrificação em sistema BFT ocasiona acúmulo de NO₃⁻ durante o ciclo de produção. Algumas estratégias podem ser utilizadas para remoção de NO₃⁻, como a denitrificação por bactérias autotróficas e/ou heterotróficas em reator externo (Hargreaves 2013, Schreier et al. 2010, Suhr et al. 2014) ou sistema associado à aquaponia (Rakocy 2012).

Esse acúmulo de substâncias (íons, NO₃⁻ e PO₄⁻) torna-se significativo em sistema BFT para a produção de tilápias do Nilo *O. niloticus*, pois o sistema utiliza alta densidade de estocagem, sendo reportada produtividade de até 45 kg/m³ (Crab et al. 2012, Luo et al. 2014). E isso representa um desafio deste sistema, devido à manutenção dos parâmetros de qualidade da água no intervalo ótimo de crescimento da espécie produzida, e as suas interações com os bioflocos. A Tabela 2 demonstra os parâmetros de qualidade de água para o cultivo de tilápia do Nilo, evidenciando a ampla tolerância das espécies aos parâmetros citados.

A tilápia do Nilo é considerada uma espécie tolerante a uma ampla faixa de pH (Mahdi 1973b, Lim & Webster 2006), por essa razão poucos estudos tem sido

realizados com esse tema, conseqüentemente não há determinação de um intervalo estreito que seja ideal para o crescimento. Além disso, os dados demonstrados por El-Sheriff & El-Feky (2009) e Rebouças et al. (2015) (Tabela 2) não podem ser considerados complementares, tão pouco definitivos. Desta forma, é necessário demonstrar a influência dos níveis de pH no crescimento de tilápias do Nilo, como também no sistema de bioflocos.

Tabela 2. Parâmetros de qualidade da água para o cultivo de tilápia do Nilo *Oreochromis niloticus*.

Parâmetro	Intervalo	Autor
	↑ Crescimento 28,0-29,0	1
Temperatura (°C)	Mínima para alimentação 18,0	2
	Mínima para sobrevivência 11,0	
Oxigênio dissolvido (mg/L)	CL50 24 h - 1,04	3
	↑ Crescimento > 3,0	4
pH	CL50 24 h - 3,4 e 11,0	5
	↑ Crescimento 7,0-8,0	6
Amônia (mg/L NH ₃)	Juvenis 4,5 g - CL50 96h - 0,96	7
Nitrito (mg/L)	Juvenis 12,5 g - CL50 96h - 81,0	8
Alcalinidade (mg/L CaCO ₃)	↑ crescimento 50,0-100,0	9
Dureza (mg/L CaCO ₃)	↑ crescimento 100,0-150,0	
Dureza:Alcalinidade	↑ crescimento 1,0-3,0	10
Salinidade (‰)	↑ crescimento 4,0-6,0	11

CL50 24 ou 96 h – concentração letal que representa a mortalidade de 50% dos indivíduos em 24 ou 96 h. “↑” - melhora. 1 - Santos et al. (2013); 2 - Atwood et al.

(2003); 3 - Mahdi (1973a); 4 - Tran-Duy et al. (2012); 5 - Mahdi (1973b); 6 - El Sheriff & El-Feky (2009); 7 - Evans et al. (2006); 8 - Atwood et al. (2001); 9 - Cavalcante et al. (2009); 10 - Cavalcante et al. (2014); 11 - Qiang et al. (2013).

O sistema BFT tem sido utilizado para as diferentes fases de produção da tilápia, demonstrando bons resultados. Como por ex., a manutenção de reprodutores nesse sistema aumenta significativamente a produção de larvas (Ekasari et al. 2015a). Além disso, a realização da larvicultura de tilápias do Nilo em sistema BFT também apresenta resultados positivos, relacionados à maior sobrevivência, aumento da resistência à infecção bacteriana e ao estresse salino (Ekasari et al. 2015b). Ainda, é relatado por Avnimelech (2012) o menor custo de produção durante a engorda de tilápia em BFT, devido ao aumento da utilização protéica microbiana e melhora na qualidade da água, reduzindo a taxa de conversão alimentar.

Entretanto, devido a alta taxa de crescimento, maior consumo dos bioflocos (Ekasari et al. 2014) e demanda de mercado, a utilização de BFT para a produção de juvenis (berçário/pré engorda) (de 0,5 até 10-100 g) é uma alternativa viável, que pode ser associada aos sistemas convencionais, como viveiros escavados ou tanques rede.

Além disso, em regiões de clima subtropical ou temperado, a produção de tilápias do Nilo fica restrita a um período entre 6-9 meses. Desta forma, durante os meses de inverno, é possível utilizar estufas com sistema BFT, tornando o meio termicamente estável, conforme demonstrado por Crab et al. (2009).

A disponibilidade de juvenis de tilápia do Nilo pode ser considerada um limitante para a produção nacional, e representa grande parte do custo de produção em tanques rede (Ayroza et al. 2011). Nessa perspectiva, a implantação de sistema BFT para berçário torna-se viável, pois utiliza estruturas menores em comparação à engorda, reduzindo o investimento.

Portanto, produzir juvenis de tilápias do Nilo em sistema BFT, é uma alternativa que atende às necessidades de sustentabilidade citadas anteriormente, como também às demandas produtivo-econômicas.

OBJETIVO GERAL

Nesse contexto, o presente estudo demonstra a correção da alcalinidade e do pH utilizando NaHCO_3 , CaCO_3 ou Ca(OH)_2 , como também os valores de pH (6,5, 7,5 e 8,3) e dureza total (controle, 250 e 700 mg/L CaCO_3), avaliando a qualidade da água, o crescimento e a sobrevivência de tilápia do Nilo *Oreochromis niloticus* durante berçário em sistema de bioflocos.

OBJETIVOS ESPECÍFICOS

- Investigar a correção da alcalinidade e do pH utilizando NaHCO_3 , CaCO_3 ou Ca(OH)_2 durante berçário de tilápia do Nilo *Oreochromis niloticus* demonstrando a qualidade da água, o crescimento, a análise proximal dos bioflocos, a hematologia e o estresse oxidativo dos peixes.
- Determinar o valor de pH ideal entre 6,5 e 8,3 durante o berçário de tilápia do Nilo *O. niloticus*, avaliando a qualidade da água, o crescimento, a análise proximal dos bioflocos, a hematologia e o estresse oxidativo dos peixes.
- Demonstrar o efeito da dureza total durante o berçário de tilápia do Nilo *O. niloticus*, avaliando a qualidade da água, o crescimento e a sobrevivência.

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ÉTICA ANIMAL

Os ensaios realizados na presente tese foram aprovados pelo Comitê de Ética Animal da Universidade Federal de Pelotas - UFPel (n° do processo 23110.001372/2016-87).

CAPÍTULO I

The Utilization of Sodium Bicarbonate, Calcium Carbonate or Hydroxide in Biofloc System: Water Quality, Growth Performance and Oxidative Stress of Nile Tilapia (*Oreochromis niloticus*)

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**The Utilization of Sodium Bicarbonate, Calcium Carbonate or Hydroxide in
Biofloc System: Water Quality, Growth Performance and Oxidative Stress of Nile
Tilapia (*Oreochromis niloticus* L.)**

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Highlights

- Utilize BFT system favoring hetero and autotrophic bacteria cause elevation on alkalizing compound consumption.
- NaHCO₃ and Ca(OH)₂ demonstrates best results for final weight, specific growth rate and net yield.
- For BFT system, the amount of alkalizing compound utilized for pH maintenance relative to feed for NaHCO₃ and Ca(OH)₂ are 14.64 ± 0.49 and 7.18 ± 0.32%, respectively.
- The alkalizing compound utilized cause increased on Ca⁺² or Na⁺ levels.
- The control of total solid suspension is the challenge for high density systems.

Abstract

In *biofloc technology* (BFT) systems, nutrient cycling for microorganisms takes place. This results in minimal or null water exchange. The sum of fish biomass and microorganisms favors alkalinity consumption and, consequently, pH reduction. It is in this context that the present study evaluates alkalinity and pH maintenance using sodium bicarbonate (NaHCO_3), calcium carbonate (CaCO_3) or calcium hydroxide (Ca(OH)_2) on BFT during a Nile tilapia *Oreochromis niloticus* nursery. In this study, 25 fishes/tank was distributed in nine experimental units (useful vol. 37.5 L) and the performance was evaluated at 60 days. During the assay, the temperature, oxygen and pH were maintained within the ideal range levels for Nile tilapia growth. All alkalizing compounds were able to pH and alkalinity correction, but when using NaHCO_3 , the alkalinity and pH were more elevated than the other treatments. Furthermore, at the beginning these assay, the total ammonia (TAN) and NO_2^- accumulate and it caused a peak, but mostly experiment remained to very low levels because of the total nitrification activity, resulting in NO_3^- accumulation. Because the non water exchange, at the final experiment the ion Na^+ accumulate when utilized NaHCO_3 , resulting in level similar to brackish water. While using CaCO_3 or Ca(OH)_2 , the Ca^{+2} ion accumulate, resulting in extremely hard water. Despite this, the fish survival was similar between treatments (about 80%). Moreover, the final weight, specific growth rate and net yield for NaHCO_3 and Ca(OH)_2 they were higher than CaCO_3 treatment. This may have been because of the higher total solid suspension (TSS) and lower protein content of the bioflocs in this treatment. In order to assess the possible physiologic alterations of the fish associated with the production system, the hematocrit, glycemia and plasmatic osmolality were evaluated. Furthermore, the antioxidant capacity against peroxy radicals (ACAP), lipid peroxidation (LPO) and catalase (CAT) and superoxide dismutase (SOD) activities on the gills and liver were also evaluated. There were no differences in biochemical/physiological parameters when the different alkalizing compounds were utilized. The results demonstrate that the use of sodium bicarbonate, hydroxide or calcium carbonate is effective on the alkalinity and pH adjustments of the final proportion of 14.64 ± 0.49 , 7.18 ± 0.32 e $24.09 \pm 2.32\%$ in relation to the feed consumption, respectively. Thus, the study demonstrates that the use of NaHCO_3 and

Ca(OH)₂ are recommended for alkalinity and pH correction during Nile tilapia nursery on BFT systems, because of the higher growth and net yield, and this sum to less amount of these compounds may represent important economic gain.

Keywords: alkalinity, pH, lime, hardness, calcium, sodium.

1. Introduction

The Nile tilapia production in *biofloc technology* (BFT) has been well developed due to high productivity, reduction in water consumption and nutrient cycling for bacteria that consumes inorganic nitrogen. For the initial formation of bioflocs, the water is fertilized with organic carbon to the proportion of carbon:nitrogen 15:1, which in turns favors heterotrophic growth (Avnimelech 1999).

Apart from heterotrophic, the BFT system permits the growth of autotrophic bacteria, or also denominated nitrifying bacteria, which are responsible for ammonia oxidation to nitrate. These microorganisms are responsible for inorganic carbon consumption on alkalinity forms (Hargreaves 2013), favoring the reduction of alkalinity and pH during the production cycle.

For the pH correction of systems with low water renovation, e.g., recirculation aquaculture systems, sodium bicarbonate (NaHCO₃) is used. When NaHCO₃ dissociates in water, a HCO₃⁻ base is produced. Besides bicarbonate, lime with limestone, which is the trade name of calcium carbonate (CaCO₃), has been traditionally used on aquaculture. This has a slow impact on the alkalinity and pH correction and, when diluted in water, reacts with CO₂, producing Ca⁺² and HCO₃⁻ (Thunjai et al. 2004). A further compound that is utilized is the calcium hydroxide or hydrated lime (Ca(OH)₂). In water, this produces the neutralization reaction: $\text{Ca(OH)}_2 + 2\text{H}^+ \rightarrow 2\text{H}_2\text{O} + \text{Ca}^{+2}$, which causes faster pH elevation, mainly when the alkalinity is low.

Both carbonate and calcium hydroxide are interesting alternatives to BFT cultivation. This is because they are both sources of Ca⁺², which is important to bioflocs for changing composition, reducing size (Luo et al. 2013) and increasing the sedimentation rate (Peeters et al. 2011). Ca⁺² is also important for Nile tilapia to balance relations between the hardness and alkalinity of the water (Cavalcante et al. 2014).

In systems, where high stocking density is applied, a strict evaluation of the biochemical and physiological characteristics would optimize the productive performance. Oxidative stress in fish has previously been evaluated as a biomarker for environmental pollution (Monserrat et al. 2007). Indeed, oxidative parameters are also considered important tools in the evaluation of challenged for the cultivation conditions that are related to the feed (Azaza et al. 2015), temperature changes (Castro et al. 2012), ammonia exposure (Hegazi et al. 2010) and other stress sources like the stocking density (Andrade et al. 2015) and pathogens (Ali et al. 2011). An oxidative stress is characterized by a situation where the antioxidant capacity is not able to compete against the reactive oxygen species (ROS) formation, affecting cellular function by protein, nucleic acids and lipids oxidation (Jones 2006).

Therefore, in order to improve the technical production of BFT, the maintenance of alkalinity is fundamental to avoid pH oscillations, providing a more stable system for bioflocs and the physiologic status of fish. The present study investigated the alkalinity and pH correction on BFT during a nursery of Nile tilapia, utilizing NaHCO_3 , CaCO_3 and Ca(OH)_2 , it evaluated the water quality, growth, proximate composition of the bioflocs, hematology and oxidative stress of the Nile tilapia juveniles.

2. Materials and Methods

2.1 Design experimental

The monosex Nile tilapia juveniles (Premium genetic) were acquired from a commercial hatchery Aquabel (Rolândia, PR, BR) and transported to “Laboratório de Piscicultura do Chasqueiro” (Universidade Federal de Pelotas, RS, BR), where the experiment took place. For acclimation, the fish were maintained on a recirculation system for 15 days until the experiment began.

During acclimation and the experiment, the Nile tilapias were fed with a commercial feed (Guabi Nutrição e Saúde Animal, BR) at 08:00, 11:00, 14:00 and 17:00 hours, according to Table 1. For the adjustment of feeding (size, crude protein (CP) and feeding rate), the biometrics were realized weekly (n = 10 fish/tank).

Table 1 Feeding of Nile tilapia *Oreochromis niloticus* juveniles during acclimation and experiment.

Feed	Wet weight (g)	Feeding rates (biomass%)	Frequency (freq/day)
1mm - 45%CP	0.5 - 5	15 - 7	4
1.7mm - 42%CP	5 - 20	6	4
2-4mm - 40%CP	20 - 50	4	4

CP – crude protein.

The assay occurred for 60 days between June and August of 2015. The treatments that were tested for alkalinity and pH correction were sodium bicarbonate - NaHCO_3 (P.A., Synth, BR), calcium carbonate - CaCO_3 (P.A., Synth, BR) and calcium hydroxide - Ca(OH)_2 (P.A., Synth, BR), which were all realized in triplicate.

For this experiment, 25 fishes/tank (666 fishes/tanks) were distributed in experimental units (useful vol. 37.5 L) with an initial weight of $3.68 \pm 0.93\text{g}$ and length 6.03 ± 0.50 cm. The tanks had two air stones on the bottom for continued aeration, heaters (100W) with a thermostat (regulated to 29°C) and a natural photoperiod. The microbial flocs that were previously produced stocked with the Nile tilapias and, were inoculated in the system (10% of the total volume). During the assay, water renovation did not occur and the weekly volume was readjusted due to evaporation and clarifications losses.

When the total ammonia (TAN) reached a value superior to 1.0 mg/L, molasses of sugar cane (45% C) addition were realized for the correction of C:N (15:1), according to the results of Avnimelech (1999). For maintenance, the total solids suspension (TSS) between 400-600 mg/L was realized and, when exceed these value, 25% of the water were removed for sedimentation on a collector of solids for 1 h. After this period, the supernatant was returned to the tank and the sediment was discarded.

The alkalinity and pH correction occurred daily. After the ultimate feeding, the amount of the alkalinizing compound was calculated according to the values of the daily pH. The final assay was the total quantity of alkalinizing compound (total AC) utilized and the consumption of this compound (relative AC%) was determined according to the formula: $\text{AC} = (\text{total chemical compound (g)} \times 100) / \text{total food (g)}$.

2.2. Water Quality and Growth Performance

The temperature and oxygen were measured twice a day (07:45 and 16:00) with a digital oxymeter (PRO 20, Yellow Springs, OH, USA). The results are expressed as the daily mean of treatments. The pH was measured at 16:00, utilizing digital pHmeter (HI 2212, HANNA Instrument, Woonsocket, RI, USA). Twice a week waters sample are collected and the total ammonia (TAN) (UNESCO 1983), nitrite (NO₂-N) and total alkalinity (APHA 1998), the total solid suspension (TSS) (Strickland and Parsons 1972), the floc volume (FV) (Imhoff cone – 30 min) and total hardness (Adad 1982) were measured. For TAN and NO₂⁻ dosages, were collected water samples before the first feeding, while the TSS, FV, alkalinity and hardness the samples were collected about the 13:30.

While the nitrate (NO₃⁻) and orthophosphate (PO₄⁻) (Aminot and Chaussepied 1983), Na⁺ (photometer flame, B462, Micronal, BR) e Ca⁺² (Doles, BR) were only measured at the final assay. The floc volume index (FVI) was determined using the following formula: $FVI = FV \text{ (mL)} / TSS \text{ (g)}$ (Yousuf 2013).

At the end of the assay, all of the fish were quantified and their weight was measured for the evaluation of survival, growth and food utilization, according to the following equations:

- a) Daily growth rate (DGR) = fish biomass increase (g) / time (days)
- b) Survival rate = $100 \times (N_f - N_i) / N_i$
- c) Net yield = fish biomass increase (g) \times 1000 / Vol (L)
- d) Feed conversion rate (FCR) = feed supply (g) / fish biomass increase (g)

Where Ni e Nf are the initial and final number of fishes.

2.3. Tissue Collection and Hematological Analyses

At the final experiment, the tissue samples (n = 6 fishes/tank) were collected for analyses. The fish were anesthetized in a benzocaine bath (50ppm) for blood, gill and liver collection. The blood was withdrawn via the arterial caudal fin (anticoagulant EDTA 10%, 1mL sterile syringe, 25 gauge needle) and centrifuged (10min, 1500 \times g) for plasma achievement. After the blood collection, the fish were euthanized for spinal

medulla rupture and then the other tissue samples were collected. The samples of the blood plasma, gill and liver were initially maintained at -180°C and after to -90°C until the analyses.

Immediately after the blood collection, glucose (Accu Chek Performa, UK) and hematocrit (15 min, 12000 RPM) were measured, while the plasma was subsequently utilized for the osmolality measurement (Vapro® Vapor Pressure Osmometer, Wescor 5600, Logan, UT).

2.4. Proximate Analyses

The proximate analyses of the bioflocs were realized on duplicate. The methodologies utilized are described for AOAC (1999). For the ash content (method access number: #942.05), the samples were burned in muffle for 4h at 600°C . The Kjeldhal method was used for the total protein determination (#984.13) after the acid digestion samples and nitrogen distillation. To calculate the protein content, the 6.5 coefficient was used. While for the ethereal extraction (#920.39), Soxhlet extraction was used for 6h, with petroleum ether as the solvent.

2.5. Biochemical Measurements

For biochemical dosages, two pools of two fish/tanks, totaling six pools, were utilized for each treatment. The samples were homogenized (1:4 w/v) in buffer (specific for each methodology), centrifuged ($20.000 \times g$, 20 min, 4°C) and the supernatant was utilized. The total protein content was determined in triplicate, utilizing the Biuret assay (Doles, BR) and was realized in a microplate reader (550 nm).

The samples for catalase (CAT) and superoxide dismutase (SOD) activities were homogenized (1:4 w/v) in buffer adjusted to pH 7.6, containing Tris base (20mM), EDTA (1mM), dithiothreitol (DTT, 1mM), sucrose (500mM), KCl (150mM) and phenylmethylsulfonyl fluoride (PMSF, 100mM).

The CAT was measured for an initial decomposition rate of 50mM H_2O_2 at 240nm (Beutler 1975). The results are expressed as CAT units, where one unit is the

enzyme amount that hydrolyzes 1 μmol of H_2O_2 for one minute and for the protein mg at 30°C and pH 8.0.

The SOD was determined based on the inhibition and auto-oxidation of epinephrine on the alkali medium of the SOD enzyme, which was monitored at 480 nm. The activity is expressed as U SOD/mg of protein at 30°C and pH 8.0, where one unit is defined as the enzyme amount that inhibits 50% of epinephrine auto-oxidation (Misra and Fridovich 1972). For SOD activity, only the liver activity was possible accomplish.

For the antioxidant capacity against peroxy radicals (ACAP) determination, the tissue were homogenized in buffer adjusted to pH 7.75, containing Tris-HCl (100mM), disodium EDTA (2mM) e $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (5mM). This was determined employing the fluorescence dye 2,7-dichlorofluorescein diacetate ($\text{H}_2\text{DCF-DA}$ – Molecular Probes) in the presence or absence of a peroxy radical generator 2, 2'-azobis 2 methylpropionamide dihydrochloride (ABAP; 4mM; Aldrich), which decomposes at 37°C producing peroxy radical (Winston et al. 1998). The difference in the fluorescence area (at excitation and emission wavelengths of 488 nm and 525 nm, respectively) with or without ABAP was considered as the antioxidant capacity measure (Amado et al. 2009).

For the lipid peroxidation (LPO) determination, the samples were homogenized (1:4 w/V) in methanol (100%, 4°C). The homogenized samples were centrifuged (1000 \times g for 10 min, 4°C) and the supernatant was employed for determination. The LPO was measured using the FOX method (Hermes-Lima et al. 1995), which is based on Fe^{+2} oxidation by lipid hidropoxides (FOX reactive substances) at an acid pH with Fe^+ complexing the xylenol orange dye. The cumene hydroperoxide was used as the standard. The results are expressed as ηmol cumene hydroperoxide/g tissue.

2.6. Statistic Analysis

Initially, the data normality and homoscedasticity were analyzed. The results were compared by ANOVA (One Way) and posterior Tukey test ($p < 0.05$). The analyses were realized on SigmaPlot 12.0 software (Systat Software, Inc., Chicago, IL). Data were presented as average \pm standard deviation.

3. Results

3.1. Water Quality

The physical and chemical parameters of the water are demonstrated in Table 2. The temperature and dissolved oxygen did not differ among the treatments. The TAN (Fig. 1a) and NO_2^- (Fig. 1b) values during the experiment did not demonstrate any differences among the treatments and, during most of the experimental period remained at very low levels. Furthermore, at the final assay, NO_3^- did not demonstrate any differences among the treatments, while the orthophosphate was more elevated for the NaHCO_3 treatment than the other treatments.

The alkalinity and pH averages (Table 2) were superior when NaHCO_3 was utilized and, in Fig. 2 are shown the daily averages of pHs along the assay. The hardness and Ca^{+2} concentration were higher for the treatments CaCO_3 or Ca(OH)_2 , while the Na^+ was superior for NaHCO_3 .

Along the experiment, TSS elevation (Fig. 3) occurred and CaCO_3 had superior values, while the FV (Table 2) was equal for all of the treatments. However, when the carbonate was utilized, the FVI was lower than the NaHCO_3 and equal to Ca(OH)_2 .

The total amount of alkalinizing compound (total AC) utilized and the relative amount (relative AC) to feed (Table 2) were superior for CaCO_3 , followed by the NaHCO_3 and Ca(OH)_2 treatments.

Table 2 Water quality in biofloc technology for Nile tilapia nursery utilizing NaHCO₃, CaCO₃ and Ca(OH)₂ for alkalinity and pH correction.

	NaHCO ₃	CaCO ₃	Ca(OH) ₂
Temperature (°C)	27.70 ± 1.17 (24.45 – 30.6)	27.55 ± 1.22 (24.95 – 31.1)	27.60 ± 1.16 (23.9 – 30.3)
O₂ (mg/L)	5.91 ± 0.61 (4.34 – 7.41)	5.93 ± 0.51 (4.55 – 7.29)	5.97 ± 0.55 (4.74 – 7.46)
pH	7.53 ± 0.02 a	7.33 ± 0.03 b	7.41 ± 0.03 ab
Total alkalinity (mg/L CaCO₃)	75.76 ± 3.78 a	48.95 ± 0.75 b	54.58 ± 6.87 b
Total hardness (mg/L CaCO₃)	101.33 ± 4.23 a	322.44 ± 34.02 b	340.44 ± 17.45 b
TSS (mg/L)	501.47 ± 37.59 a	707.49 ± 49.34 b	577.77 ± 43.43 a
FV (ml/L)	49.75 ± 10.56	44.37 ± 10.67	47.50 ± 4.75
FVI (mL/g)	71.47 ± 21.68 a	41.34 ± 16.75 b	62.54 ± 16.75 ab
TAN (mg/L)	1.17 ± 3.50	0.73 ± 2.69	1.04 ± 3.38
NO₂ (mg/L)	7.03 ± 10.61	5.94 ± 10.59	8.14 ± 13.65
NO₃⁻ (mg/L)	140.00 ± 34.64	117.50 ± 10.60	143.33 ± 14.43
PO₄⁻ (mg/L)	8.3 ± 0.51 a	1.75 ± 0.49 b	3.6 ± 1.9 b
Na⁺ (mg/L)	597.05 ± 279.29 a	66.46 ± 14.30 b	72.43 ± 38.58 b
Ca⁺² (mg/L)	58.54 ± 5.17 a	239.73 ± 25.10 b	299.15 ± 63.99 b
Total AC (g)	120.46 ± 6.05 a	186.56 ± 3.51 b	55.6 ± 3.83 c
Relative AC (%)	14.64 ± 0.49 ab	24.09 ± 2.32 b	7.18 ± 0.32 a

Data represents mean \pm S.D (Tukey, $p < 0.05$). Between parenthesis are maximum and minimum values. TSS – total solid suspension; FV – floc volume; FVI – floc volume index; TAN – total ammonia; AC – alkalizing consumption.

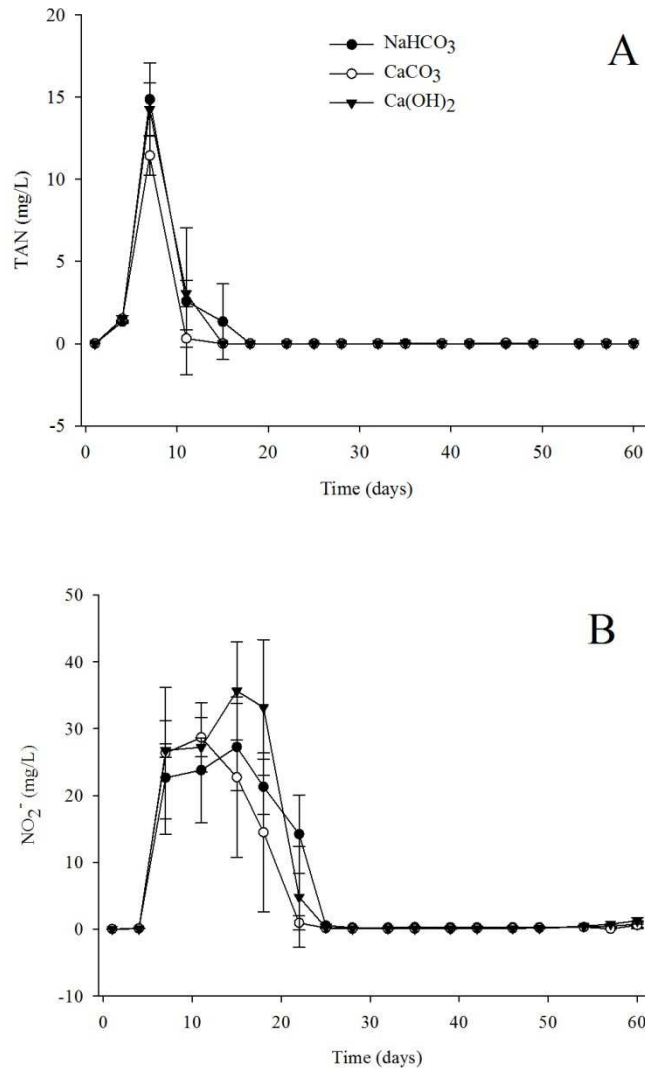


Fig. 1. Mean of total ammonia (TAN) (A) and NO₂⁻ (B) on the culture of Nile tilapia *Oreochromis niloticus* in biofloc technology utilizing different chemical compounds for alkalinity correction. Data are mean \pm SD of three replicate tanks per sampling time.

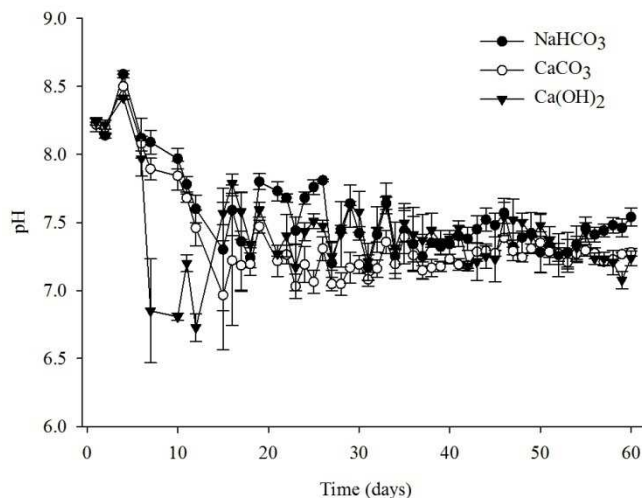


Fig. 2. pH values on the culture of Nile tilapia *Oreochromis niloticus* in biofloc technology utilizing different chemical compounds for pH correction. Data are mean \pm SD of three replicate tanks per sampling time.

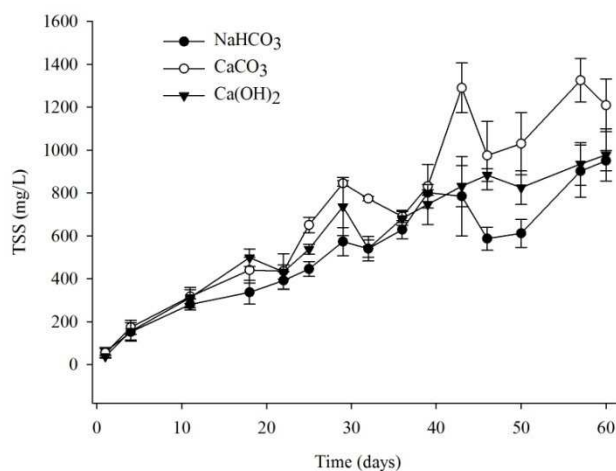


Fig. 3. Total suspended solids (TSS) on the culture of Nile tilapia *Oreochromis niloticus* in biofloc technology utilizing different chemical compounds for alkalinity correction. Data are mean \pm SD of three replicate tanks per sampling time.

3.2. Growth and Proximate Analyses

The growth results are shown in Table 3. The final weight, specific growth rate and net yield were higher for NaHCO₃, but statistically equal to Ca(OH)₂. This treatment also has final weight, specific growth rate and net yield statistically equal to

CaCO₃. Meanwhile, the final length, survival and food conversion rate did not differ among the treatments.

Table 3 Growth performance and feed utilization of Nile tilapia raised on biofloc technology utilizing NaHCO₃, CaCO₃ and Ca(OH)₂ for pH and alkalinity correction. Fishes (3.68 ± 0.93 g and 6.03 ± 0.50 cm) are stocked in 37.5 L tanks at 25 fish/tank, for 60 days.

	NaHCO ₃	CaCO ₃	Ca(OH) ₂
Final weight (g)	44.09 ± 0.93 a	38.29 ± 1.29 b	40.57 ± 1.67 ab
Final length (cm)	13.16 ± 0.08	13.07 ± 0.32	13.04 ± 0.12
DGR (g/day)	0.67 ± 0.01 a	0.57 ± 0.02 b	0.61 ± 0.02 ab
Survival rate (%)	80.00 ± 17.43	81.33 ± 12.85	80 ± 10.58
Net yield (kg/m³)	23.52 ± 0.49 a	20.76 ± 0.70 b	21.64 ± 0.89 ab
FCR	1.18 ± 0.07	1.10 ± 0.16	1.13 ± 0.10

Each value represents mean ± S.D (Tukey, *p* < 0.05). SGR – specific growth rate, FCR – food conversion rate.

The proximate composition of bioflocs is represented in Table 4. The protein and ash content was more elevated for the bicarbonate and calcium hydroxide treatments, while the lipid content did not differ among the treatments.

Table 4 Crude protein (CP), crude lipid (CL) and ash (dry basis %) of biofloc maintained at different source correction of pH and alkalinity.

	NaHCO ₃	CaCO ₃	Ca(OH) ₂
CP	16.76 ± 0.38 a	13.20 ± 0.95 b	17.85 ± 1.02 a
CL	2.11 ± 0.29	2.65 ± 0.45	2.63 ± 0.33
Ash	42.82 ± 3.25 a	35.02 ± 0.99 b	44.03 ± 3.63 a

Each value represents mean ± S.D (Tukey, *p* < 0.05).

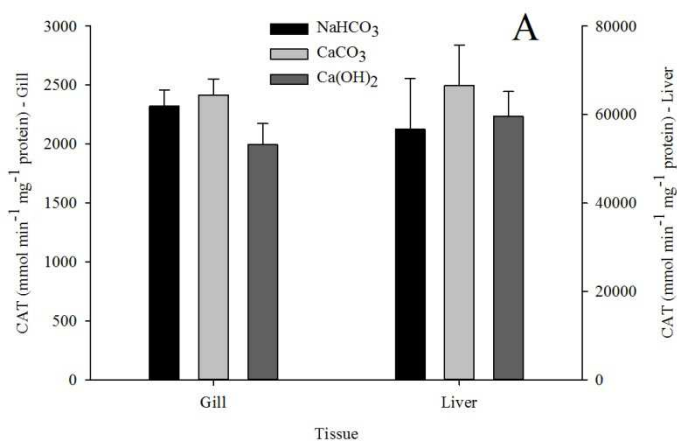
3.3. Hematological Analyses and Stress Oxidative

The hematological parameters of glucose, hematocrit and osmolality (Table 5) did not demonstrate alterations and were equal among the treatments. Similarly, the CAT (Fig. 4 A) and SOD (Fig. 4 B) activities, as well the ACAP (Fig. 5) and LPO (Fig. 6), did not differ among the treatments.

Table 5 Hematologic analyses of tilapia juveniles cultured at different source correction of pH and alkalinity on biofloc system.

	NaHCO_3	CaCO_3	Ca(OH)_2
Glucose (mg/dL)	39.50 ± 3.62	35.00 ± 3.18	37.50 ± 3.50
Hematócrito (%)	32.33 ± 1.13	27.00 ± 3.18	29.00 ± 1.27
Osmolality (mOsm)	316.66 ± 9.81	315.21 ± 9.81	312.00 ± 10.07

Each value represents mean \pm S.D ($p < 0.05$; $n = 6$).



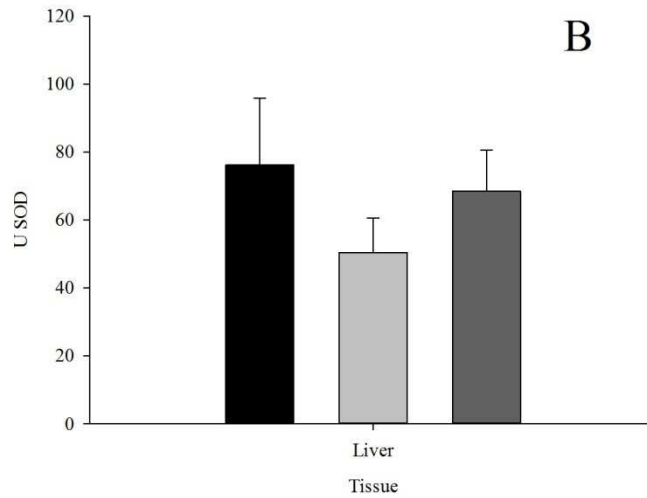


Fig. 4. Catalase (CAT) (A) and superoxide dismutase (SOD) (B) activity in gills and liver of Nile tilapia *Oreochromis niloticus* raised at different alkalizing compound for pH and alkalinity correction.(mean \pm SD; $p < 0.05$; n = 6).

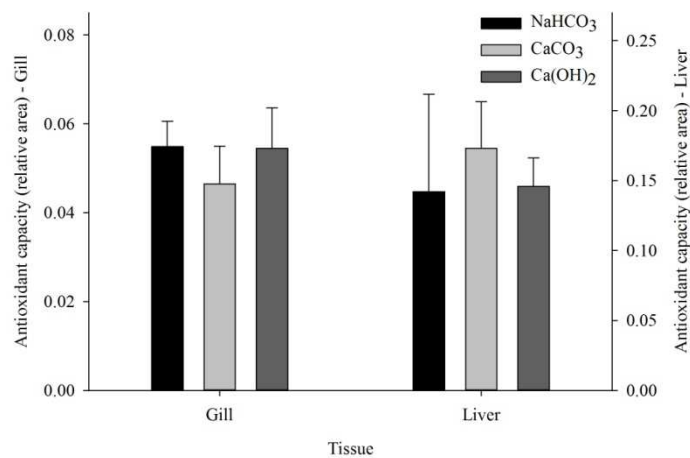


Fig. 5. Antioxidant capacity against peroxy radicals (relative area) in gills and liver of Nile tilapia *Oreochromis niloticus* raised at different compound chemical for correction alkalinity. (mean \pm SD; $p > 0.05$; n = 6).

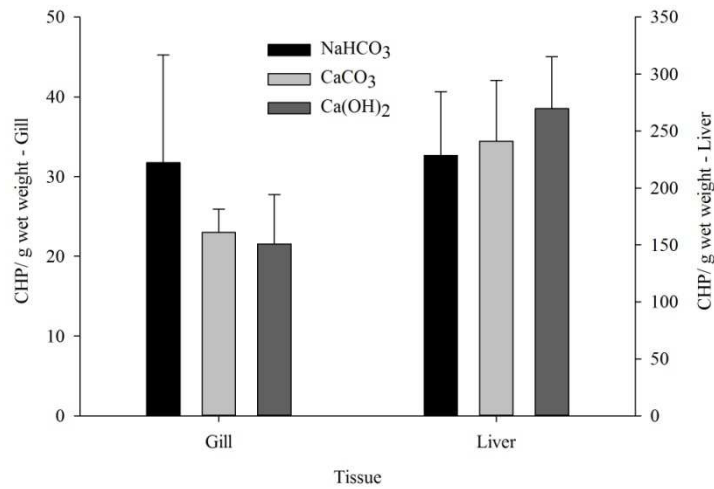


Fig. 6. Lipid Peroxidation in gills and liver of Nile tilapia *Oreochromis niloticus* raised at different compound chemical for correction alkalinity. (mean \pm SD; $p > 0.05$; $n = 6$).

4. Discussion

During the assay, the water temperature (28-29°C) and dissolved oxygen (>3mg/L) levels remained at a rate that is considered ideal for Nile tilapia *O. niloticus* growth (Santos et al. 2013, Tran-Duy et al. 2012). Furthermore, all the treatments were within the pH range that is considered ideal for the species, between 7 and 8 (El-Sayed 2006, El-Sherif and El-Feky 2009). This suggests that the tested alkalinizing compounds are efficient for alkalinity and pH correction.

At the beginning of the experiment, the sudden elevation of TAN and NO_2^- occurred in all of the treatments. According to Emerson et al. (1975), that demonstrated the relation between pH and temperature, the maximum values of non-ionized ammonia (NH_3) in present study for the NaHCO_3 , Ca(OH)_2 or CaCO_3 were 0.98, 0.96 e 0.74 mg/L, respectively. These averages are nearby to lethal concentration (CL50-96h), estimated in 0.96 mg/L of NH_3 for Nile tilapia juveniles – 12.6 g (Evans et al. 2006). However, in this study, TAN reduction quickly occurred and, remained very low during most of the experiment.

Still, the NO_2^- values reached the maximum value of 35.66 mg/L when calcium hydroxide was used. However, this is inferior to CL50-96h, which Atwood et al. (2001) demonstrated is 81 mg/L for Nile tilapia juveniles – 4.4 g. NO_2^- toxicity has mainly

been associated with hematocrit and hemoglobin reduction, besides of methemoglobin formation (Yildiz et al. 2006), which impair oxygen transport. This, in addition to the TAN increase, was probably the reason for mortality in all of the treatments, verified at the beginning of the assay. Nevertheless, the survival in this experiment is very satisfactory, about the 80% for all treatments.

The fertilizations with molasses were realized when the TAN reached values less than 1.0mg/L, favoring the heterotrophic bacteria's growth. After the TAN reduction, the fertilization was finalized, initiating the nitrification process. During this process the partial oxidation of ammonia occurred, producing NO_2^- , as verified between 11 and 15 days of the assay.

The total hardness at the final assay was very elevated for the CaCO_3 and Ca(OH)_2 treatments. According to Boyd (1990), the medium values for these treatments are considered extremely hard, while, for NaHCO_3 , they are moderately hard. However, in this study, the total hardness does not seem to have influenced the growth, as the calcium hydroxide treatment demonstrated the same performance as the sodium bicarbonate. Cavalcante et al. (2012) demonstrates significant improvement on growth of Nile tilapia juveniles to elevation of hardness from about 55 to 150 mg/L CaCO_3 . But further studies are needed to relate the high values of hardness and Ca^{+2} to the growth of *O. niloticus*.

The formation, structure, stability and size of the bioflocs are dependents of water ionic composition (De Schryver et al. 2008). Previous studies have demonstrated that a higher Ca^{+2} concentration increases the biofloc density, favoring sedimentation (Luo et al. 2013, Peeters et al. 2011). This is directly related to the size and FVI of the bioflocs, which have ideal values between 40-60 mL/g (De Schryver et al. 2008). In this study, the CaCO_3 treatment demonstrated lower FVI values than the other treatments, which represents a higher density and sedimentation velocity.

In addition to Ca^{+2} , the Na^+ also has an important influence on the composition and structure of the flocs, neutralizing the negative charges of the particles, favoring the adhesion (Peeters et al. 2011). The Na^+ concentration for the NaHCO_3 treatment was higher than the other treatments, reaching 597.05 ± 279.29 mg/L. Although some studies have demonstrated the brackishwater (6-8‰) benefit on growth of Nile tilapia (Jun et al. 2012, Qiang et al. 2013), this Na^+ concentration was not enough to improve

the growth, because the growth performance was similar to Ca(OH)_2 , reaching Na^+ concentration of 72.43 ± 38.58 mg/L.

The TSS control is considered limiting for the high density system and, previous studies with BFT have demonstrated TSS values superior of 500 mg/L (Avnimelech 2007, Azim and Little 2008, Long et al. 2015). Our results showed that, in the last 15 days, the TSS was superior for CaCO_3 , reaching values that were superior of 1000 mg/L. This may be associated with the chemical characteristics of the alkalizing compound, because a greater total amount of CaCO_3 (186.56 ± 3.51 g) was used compared to Ca(OH)_2 (55.6 ± 3.83 g).

When NaHCO_3 was used, the growth and final density was equal to the Ca(OH)_2 and, it was higher compared to the CaCO_3 . The lesser growth for CaCO_3 can be attributed to the sharp rise in the TSS, because high concentration of TSS is associate to gill clogging, causing difficulty on gas and ions exchanges through the gills (Hargreaves 2013). Moreover, when using NaHCO_3 , the total hardness:alkalinity relation was more proximate to those of Cavalcante et al. (2012), who demonstrated an improvement in the growth of Nile tilapia juveniles, while keeping the ratio at 1:1.

There can be advantages to utilizing the BFT system, favoring the mixed microbiologic system (hetero and autotrophic bacteria). For example, the lower C organic consumption and the TSS produced, as well as the reduction in O_2 and alkalinity consumptions (Ebeling et al. 2006, Browdy et al. 2012). The economy caused by reducing those elements may represent a significant reduction in the production cost. In this context, Ray and Lotz (2014) compared the performance of white shrimp *Litopenaeus vannamei* on BFT utilizing a hetero and chemoautotrophic system. They confirmed that the second system leads to a reduction in oxygen consumption and lower TSS production, without a loss of growth.

In the present study, there was high consumption of alkalizing compound. Due to the chemical characteristics of each compound, there were differences in consumption, which influenced the proportion that was utilized in relation to the feed. The calcium hydroxide had a capacity to increase the pH quickly, mainly when there was low alkalinity. Therefore, it can be said that it must be used with caution. Ganguly et al. (1999) reported these characteristics when they utilized 0.1-0.2 g/L. They assign the bactericidal capacity of stress to the quick increase in pH. Our results did not

demonstrate any damage to the bioflocs or fish due to the calcium hydroxide, using the maximum daily concentration of 0.048 g/L. This was a less value than that reported by Furtado et al. (2011), when 0.15 g/L lime was employed without causing damage to bioflocs and white shrimp *L. vannamei*.

The PO₄-P levels on the final assay remained low when the CaCO₃ or Ca(OH)₂ was utilized. The reaction between Ca⁺² and PO₄-P, producing calcium phosphate (Ca₃(PO₄)₂) and mineral phosphate (Li et al. 2012), was probably responsible for the lower levels of treatment with high Ca⁺² concentration. Luo et al. (2014) also obtained low PO₄-P on yours results, however they attributed the low values to the assimilation of microorganisms present on the bioflocs, suggesting the cycling of phosphorus in addition to nitrogen. This immobilization/cycling of PO₄-P on medium is an important result, because 80-90% of the phosphorus utilized in the feed is released in the production system (Barak et al. 2003).

This is the best utilization of nutrients on BFT occur due to the absorption for the organisms that compose the bioflocs and, the harvesting of Nile tilapia. Ekasari et al. (2014) demonstrated that juveniles of Nile tilapia (9.6 g) consume around 90 g TSS/kg wet weight. From this perspective, the biofloc represents an extra source of protein, lipid, carbohydrate and energy.

The protein content of bioflocs in all treatments presented values below those previously reported by Azim and Little (2008) and Lopes-Elias and Moreno-Arias (2015). However, the ash content was more elevated and previous researches with Nile tilapia have demonstrated ash values between 7-40% (Azim et al. 2007, Ekasari et al. 2010, Lopes-Elías and Moreno-Arias 2015). These results probably occurred because of the intense harvesting of Nile tilapia and, besides that the utilization of the feed with a higher total protein. These two factors result in a lower C:N relation in the system, favoring the development of autotrophic bacteria, which are responsible for the high consumption of alkalizing compound and, consequently results in a higher ash content.

The hematological index and biochemical evaluations gave insights into the health status of the fish. The glucose and hematocrit did not demonstrate any differences among the treatments and were within the range that is considered normal for Nile tilapia (Bittencourt et al. 2003). These hematological parameters associated with none

alteration in antioxidant capacity evaluation can be reflective of the favorable conditions.

The SOD and CAT enzymes act in a highly coordinated system. The alteration in the activity of both enzymes is related to the conditions of the environment that causes oxidative stress, such as management (Braun et al. 2010), hypoxia (Welker et al. 2013) and ammonia (Sinha et al. 2014). The SOD is part of the antioxidant system and, is responsible for the conversion of superoxide anions O_2^- on less toxic products H_2O_2 and O_2 (Gaté et al. 1999). The CAT catalyzes the removal of H_2O_2 , transforming in water and molecular oxygen. In the present study, there were no differences in the CAT and SOD activities. Furthermore, there were no alterations on the antioxidant capacity and lipidic peroxidation. Thus, our results demonstrated similar and favorable conditions for growth when using different alkalinizing compounds.

5. Conclusions

The sodium bicarbonate utilization, calcium hydroxide or carbonate are effectives on the alkalinity and pH correction on BFT systems. Due to the lower capacity of calcium carbonate of correct the pH and alkalinity, there was utilized higher amount from these compound. This significantly contributed to the increase in the TSS, which reached superior values of 1000 mg/L and, high TSS associated with a low protein content of bioflocs, which could be responsible for the diminished growth in these treatments. However, when evaluating the hematological index and oxidative stress, the alterations were not verified, indicating similar conditions among the treatments. Thus, the best alternatives for alkalinity and pH correction are sodium bicarbonate or calcium hydroxide.

More research is necessary in order to evaluate the water quality, mainly related to the interaction and efficiency of bacteria, both autotrophics and heterotrophics, as also ionic composition of reuse water for seeking the maximum efficiency of growth in BFT system.

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CAPÍTULO II

Growth, Water Quality and Oxidative Stress of Nile Tilapia *Oreochromis niloticus* (L.) in Biofloc Technology System at Different pH

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Growth, Water Quality and Oxidative Stress of Nile Tilapia *Oreochromis niloticus* (L.) in Biofloc Technology System at Different pH

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Highlights

- It is beneficial for nitrification rate in the BFT system to start with pH of about the 8.0.
- Growth of Nile tilapia in the BFT system utilizing pH 6.5–7.5 is the best alternative.
- pH 6.5 produces less solid suspension than higher pH values.
- Reducing pH can result in low ionic alteration during production.

Abstract

Biofloc technology (BFT) is a sustainable alternative to Nile tilapia production, mainly because of the nitrogen waste cycling that result in minimal water exchange. The management of BFT, favoring the hetero and autotrophic bacterial growth, causes high

alkalinity consumption and consequent pH reduction. Previous research has demonstrate that nitrification rate influences pH, therefore, to keep it at appropriate levels, current practice involves adding alkalizing compounds, such as NaHCO_3 to the system. In this context, this study demonstrates the influence of pH on growth, survival, water quality, on proximal composition of bioflocs and on oxidative stress of Nile tilapia in the BFT system. Twenty-five fishes (3.68 ± 0.93 g) were distributed in each tank (useful vol. 37.5 L), utilizing treatments in triplicate with medium levels of pH 6.5, 7.5 and 8.3 at 60 days. During the assay, the oxidation of total ammonia (TAN) to NO_2^- was similar among the treatments. However to the oxidation of NO_2^- was slower at pH 6.5, with superior mean (10.1 ± 1.0 mg/L), compared to pH 7.5 (7.0 ± 0.6 mg/L) and 8.3 (7.1 ± 1.5 mg/L). After the total oxidation of NO_2^- to NO_3^- , does not was pH influence about the nitrification. At the final experiment the fishes from the 7.5 treatment reached higher final weight (44.1 ± 0.9 g) compared to pH 8.3 (37.1 ± 3.9 g). At pH 6.5 the final weight was similar to that observed with other treatments (40.4 ± 4.1 g). In contrast, the survival, specific growth rate (SGR) and the food conversion rate (FCR) were not affected by treatments. Moreover, the bioflocs at pH 6.5 demonstrate more crude protein and ash content when compared to other treatments. When evaluating glucose, hematocrit and osmolality, the results were similar among the treatments and they are within the range considered normal for Nile tilapia. Further, to assess the oxidative stress for antioxidant capacity against peroxy radicals (ACAP), lipidic peroxidation (LPO) and superoxide dismutase (SOD) and catalase (CAT) activity, no alterations were detected. This result indicates that pH in the range of 6.5 to 8.3 does not cause hematologic or oxidative damage. Thus, the present study demonstrates that BFT for a Nile tilapia nursery, utilizing the range of pH 6.5 to 7.5 after the establishment of complete nitrification, promotes the best results in terms of growth and productivity.

Keywords: ions, nursery, heterotrophic, autotrophic, solid suspension

1. Introduction

Biofloc technology (BFT) represents an alternative to Nile tilapia production, with advantages related to broodstock maintenance (Ekasari et al. 2015a) and larval

production (Ekasari et al. 2015b), as well as improved of digestive enzymes activities and immune function (Long et al. 2015). The BFT uses low water renovation and high stocking density, because of nitrogen (N) inorganic cycling, which is performed for a variety of organisms, such as bacteria, microalgae, fungi, zooplankton and protozoa is associate with extracellular polymeric substances (EPS) and organic material (De Schryver et al. 2008).

In BFT, bacteria are mainly responsible for nitrogen waste cycling, and basically two primary groups compose the bioflocs: heterotrophics and autotrophics bacteria. To add organic carbon to the system, the heterotrophic bacteria would assimilate ammoniacal nitrogen quickly, producing microbial biomass (Avnimelech 1999). Due to fast growth, this group is responsible for high production and the accumulation of solids in the system, aside from oxygen oscillations due to this significant consumption (Hargreaves 2006, De Schryver and Verstraete 2009). Different strategies for organic fertilization have been proposed for biofloc formation on Nile tilapia production, for example the utilization of different C:N (Pérez-Fuentes et al. 2016) ratios and carbon sources (Ekasari et al. 2010).

In contrast, the autotrophic bacteria utilize inorganic carbon, mainly from of alkalinity, and realize the nitrification process in two steps. First the ammonium is oxidized to nitrite and after to nitrate, which is a less toxic nitrogen compound (Burton and Prosser 2001). After the establishment of complete nitrification, the process is reliable, however, the establishment and growth of bacterial groups can be inconsistent and slow, causing oscillations mainly related to nitrite oxidation. The nitrification is generally utilized in recirculating aquaculture systems (RAS), being used substrates for adhesion of the bacterial groups (Timmons et al. 2010). The main advantages of autotrophic bacteria are the less solid production and reduced oxygen consumption, when compared to heterotrophics (Ebeling et al. 2006).

The nitrification rate is influenced by N inorganic level, bacterial biomass, temperature, oxygen and pH (Chen et al. 2006). Therefore, the water quality control is very important, and various studies were performed on systems for effluent treatments and bioreactors, to test pH levels in order to evaluate the nitrification rate. Varied results are found in the literature; in his review Gujer (2010) quotes a range of pH from 6.2 to

8.0, while Jimenez et al. (2011) demonstrates that a range between 7.0 and 8.0 is ideal for nitrification in a reactor of activated sludge.

Besides harming to the functioning of BFT system operation, the pH oscillations can represent physiologic challenges to fishes. The decrease of pH has been related to disturbances in acid-base balance, increased of gill permeability and consequent ionic losses and water absorption (Wood 2001), besides the alteration on ammonia excretion (Golombieski et al. 2013) and oxidative stress (Mai et al. 2010).

Previous studies on BFT systems for Nile tilapia have reported alkalinity consumption and pH reduction (Azim and Little 2008; Pérez-Fuentes et al. 2016), however, no studies testing these variables for BFT have been performed. The utilization of systems with pH below of the neutrality can be favorable to Nile tilapia growth, and it is has been shown that growth improves in ponds utilizing pH between 4–6 (Rebouças et al. 2015). However, as previously mentioned, the pH below of 7.0 may compromise the nitrogen cycling. Thus, for pH maintenance, the utilization of alkalizing compound is necessary. Considering the mentioned above, this study aims to investigate the BFT utilization with pH 6.5, 7.5 and 8.3, evaluating the growth, water quality, proximate composition, hematology and oxidative stress of Nile tilapia in a nursery on the BFT system.

2. Materials and methods

2.1. Management and experimental design

The monosex Nile tilapia juveniles (Premium genetic) acquired from Aquabel commercial hatchery (Rolândia, PR, BR), and transported to “Laboratório de Piscicultura do Chasqueiro (Universidade Federal de Pelotas, RS, BR), where assay occurred. For acclimation, the fishes were maintained in recirculation aquaculture system for fifteen days, until the experiment initiate. During acclimation and assay, the Nile tilapias were fed with commercial feed (Guabi Nutrição e Saúde Animal S.A., BR) on equal amounts at 08:00, 11:00, 14:00 and 17:00 h (Tab 1).

The assay lasted for 60 days. Twenty five fishes (3.68 ± 0.93 g) were distributed for each experimental unit (useful vol. 37.5 L). The tanks had two air stones in bottom for continuous aeration and were heated (100W) with thermostat (adjusted to 28°C).

Table 1 Feeding of tilapia juveniles *Oreochromis niloticus* during acclimation and experiment.

Feed (size mm – % CP)	Fish wet weight (g)	Feeding rates (biomass %)	Frequency (freq.day⁻¹)
1 – 45	0.5 - 5	15 - 7	4
1.7 - 42	5 - 20	6	4
2-4 - 40	20 - 50	4	4

CP – crude protein.

Microbial floc previously produced in Nile tilapia tanks was inoculated in system at 10% of total volume. The treatments consist of pH 6.5, 7.5 and 8.3, all realized in triplicate, employing NaHCO₃ as alkalizing compound. In order to calculate pH average throughout assay, it was considered the beginning at tenth day, when all experimental unities reached the nominal pH value.

When the total ammonia (TAN) reached values up to 1.0 mg/L, the addition of sugar cane molasses (\approx 45% C) was realized for C:N ratio correction (15:1), according to Avnimelech (1999). For maintenance of total solid suspension (TSS) in values between 400-600 mg/L, biofloc removals were realized employing sedimentation of 25% total volume on a collector of solids for 1 h. After this period, the supernatant was returned to tank and the sludge was discharged. Water exchange was not applied during assay and the total volume was readjusted weekly, due to losses with clarifications and evaporation.

The alkalinity and pH correction occurred daily after the last feeding, and calculated according to daily pH values. At final assay, the amount of feed and alkalizing compound was quantified. The relative consumption of alkalizing (relative AC%) was determined according to the formula: $AC = (\text{total chemical compound (g)} \times 100) / \text{total food (g)}$.

2.2. Water quality and growth performance

The temperature and dissolved oxygen were measured twice a day (07:45 and 16:00 h), utilizing YSI PRO 20 (YSI Incorporated, Yellow Springs, OH, USA). The pH was daily measured (16:15 h) with digital pHmeter (HI 2212, HANNA Instrument, Woonsocket, RI, USA). Twice a week, TAN (UNESCO 1983), NO_2^- , total alkalinity (APHA, 1998), total solid suspension (Strickland and Parsons 1972), floc volume (FV) (Imhoff Cone – 30 min) and total hardness (Eaton et al. 2005) were dosed. For TAN and NO_2^- the samples were collected after the first feeding. NO_3^- and PO_4^- (Aminot and Chaussepied 1983), Na^+ (photometer flame, B462, Micronal, BR) and the Ca^{+2} (Arsenazo calcium, Doles, BR), were dosed only at the end of assay. The floc volume index was determinate according following formula: $\text{FVI} = \text{FV (mL)} / \text{SST (g)}$ (Yousuf 2013).

Weekly biometrics (n=10) (fishes were sedated with benzocaine 50ppm) were realized, for feed adjustment, according to Table 1. At final assay, weight were quantified for all fishes for evaluation of following parameters:

- a) Daily growth rate (g/day) = fish biomass increase (g) / time (days)
- b) Survival rate (%) = $100 (\text{Nf}-\text{Ni}) / \text{Ni}$
- c) Net yield = fish biomass increase (g) *1000 / Vol (L)
- d) Feed conversion rate (FCR) = feed supply (g) / fish biomass increase (g)

Where Ni e Nf are initial and final number of fishes.

2.3. Tissue collection and hematological analyses

Tissue samples (n = 6 fishes/tank) were collected for analyses at the end of experimental period. The fishes were anesthetized with benzocaine (50ppm) for blood collection. The blood was withdrawn via the arterial caudal fin (anticoagulant EDTA 10%, 1mL sterile syringe, 25 gauge needle) and centrifuged (10min, $1500 \times g$) for plasma collections. After the blood collection, the fish were euthanized for spinal medulla rupture and then tissue samples (gill and liver) were collected. The samples of the blood plasma, gill and liver were initially maintained at -180°C and after to -90°C until the analyses.

After blood collection, glucose (Accu Chek Performa, UK) and hematocrit (15 min, 12000 RPM) were measured, while the plasma was subsequently utilized for the osmolality measurement (Vapro® Vapor Pressure Osmometer, Wescor 5600, Logan, UT).

2.4. Proximate Analyses

The proximate analyses of the bioflocs were realized on duplicate. The methodologies utilized are described for AOAC (1999). For the ash content (method access number: #942.05), the samples were burned in muffle for 4h at 600°C. The Kjeldhal method was used for the total protein determination (#984.13) after the acid digestion samples and nitrogen distillation. To calculate the protein content, the 6.5 coefficient was used. While for the ethereal fraction extraction (#920.39), extraction in Soxhlet was used for 6h, with petroleum ether as the solvent.

2.5. Biochemical Measurements

For biochemical dosages, two pools of two fish/tanks, totaling six pools, were utilized for each treatment. The samples were homogenized (1:4 w/v) in buffer (specific for each methodology), centrifuged ($20.000 \times g$, 20 min, 4°C) and the supernatant was utilized. The total protein content was determined in triplicate, utilizing the Biuret assay (Doles, BR) in a microplate reader (550 nm).

The samples for catalase (CAT) and superoxide dismutase (SOD) activities were homogenized (1:4 w/v) in buffer adjusted to pH 7.6, containing Tris base (20mM), EDTA (1mM), dithiothreitol (DTT, 1mM), sucrose (500mM), KCl (150mM) and phenylmethylsulfonyl fluoride (PMSF, 100mM).

The CAT was measured for an initial decomposition rate of 50mM H₂O₂ monitored spectrophotometrically at 240nm (Beutler 1975). The results are expressed as CAT units, where one unit is the enzyme amount that hydrolyzes 1µmol of H₂O₂ for one minute and for the protein mg at 30°C and pH 8.0.

The SOD was determined based on the inhibition of epinephrine auto-oxidation on the alkali medium by SOD enzyme, which was monitored at 480 nm. The activity is

expressed as U SOD at 30°C and pH 8.0, where one unit is defined as the enzyme amount that inhibits 50% of epinephrine auto-oxidation per mg of protein (Misra and Fridovich 1972). For SOD activity, only the liver activity was possible accomplish.

For the antioxidant capacity against peroxy radicals (ACAP) determination, the tissue were homogenized in buffer adjusted to pH 7.75, containing Tris-HCl (100mM), disodium EDTA (2mM) e MgCl₂.6H₂O (5mM). This was determined employing the fluorescence dye 2,7-dichlorofluorescein diacetate (H₂DCF-DA – Molecular Probes) in the presence or absence of a peroxy radical generator 2, 2'-azobis 2 methylpropionamide dihydrochloride (ABAP; 4mM; Aldrich), which decomposes at 37°C producing peroxy radical (Winston et al. 1998). The difference in the fluorescence area (at excitation and emission wavelengths of 488 nm and 525 nm, respectively) with or without ABAP was considered as the antioxidant capacity (Amado et al. 2009).

For the lipid peroxidation (LPO) determination, the samples were homogenized (1:4 w/V) in methanol (100%, 4°C). The homogenized samples were centrifuged (1000 × g for 10 min, 4°C) and the supernatant was employed for determination. The LPO was measured using the FOX method (Hermes-Lima et al. 1995), which is based on Fe⁺² oxidation by lipid hidroperoxides (FOX reactive substances) at an acid pH with Fe⁺ complexing dye xylenol orange. The cumene hydroperoxide was used as the standard. The results are expressed as ηmol cumene hydroperoxide/g tissue.

2.6. Statistic Analysis

Previously, the data normality (Shapiro-Wilk) and homoscedasticity were analyzed. The results were compared by ANOVA (One Way) and posterior Tukey test (p<0.05). The analyses were realized on SigmaPlot 12.0 software (Systat Software, Inc., Chicago, IL). Data were presented as average ± standard deviation.

3. Results

The results of water quality are presented in Tab. 2. The average temperature and dissolved oxygen do not differ among treatments. According the proposed, the

medium values of pH differ among the treatments, with medium values of 6.56 ± 0.02 , 7.49 ± 0.02 and 8.27 ± 0.02 . The daily values of pH and alkalinity throughout the assay are demonstrated on Fig. 1A and 1B, respectively. The final mean of total alkalinity did not differ among the 6.5 and 7.5 treatments, but were superior for pH 8.3. Total hardness did not differ among the treatments.

Table 2 Medium values of water quality on tilapia nursery in biofloc system utilizing different pH.

	6.5	7.5	8.3
Temperature (°C)	27.55 ± 1.12 (25.50 - 31.00)	27.70 ± 1.17 (24.45 - 30.60)	27.60 ± 1.28 (24.25 - 31.40)
O₂ (mg/L)	6.04 ± 0.57 (4.56 - 7.56)	5.91 ± 0.61 (4.34 - 7.41)	5.69 ± 0.67 (3.83 - 7.48)
pH	6.56 ± 0.02 a	7.49 ± 0.02 b	8.27 ± 0.02 c
TAN-N (mg/L)	1.68 ± 0.44	1.25 ± 0.44	0.94 ± 0.09
NO₂-N (mg/L)	10.10 ± 1.08 a	7.03 ± 0.62 b	7.15 ± 1.56 b
NO₃-N (mg/L)	105.00 ± 8.66	140.00 ± 34.64	123.33 ± 34.03
PO₄-P	10.23 ± 1.43	8.3 ± 0.51	6.93 ± 1.00
Total alkalinity (mg/L CaCO₃)	44.72 ± 7.54 a	75.76 ± 3.78 a	175.90 ± 24.56 b
Total hardness (mg/L CaCO₃)	95.11 ± 4.23	101.33 ± 4.23	72.00 ± 3.21
TSS (mg/L)	485 ± 39.23	501.47 ± 37.59	548.61 ± 24.48
FV (mL/L)	23.87 ± 2.67 a	49.75 ± 10.56 b	39.75 ± 2.45 ab
FVI (mL/g)	31.89 ± 12.46 a	71.47 ± 21.68 b	65.61 ± 17.65 ab
Na⁺ (mg/L)	$456.78 \pm$ 214.49	597.05 ± 279.29	657.75 ± 305.62

Ca⁺² (mg/L)	72.67 ± 30,28 a	58.54 ± 5,17 a	46.23 ± 3.44 b
Total AC (g)	94.06 ± 1.85 a	120.46 ± 6.05 b	163.70 ± 8.69 c
Relative AC (%)	12.83 ± 0.7 a	14.64 ± 0.49 a	19.64 ± 0.92 b

Data represents mean ± S.D (Tukey, $p < 0.05$). Between parenthesis are maximum and minimum values. TAN – total ammonia; TSS – total solid suspension; FV – floc volume; FVI – floc volume index; AC – alkalizing consumption.

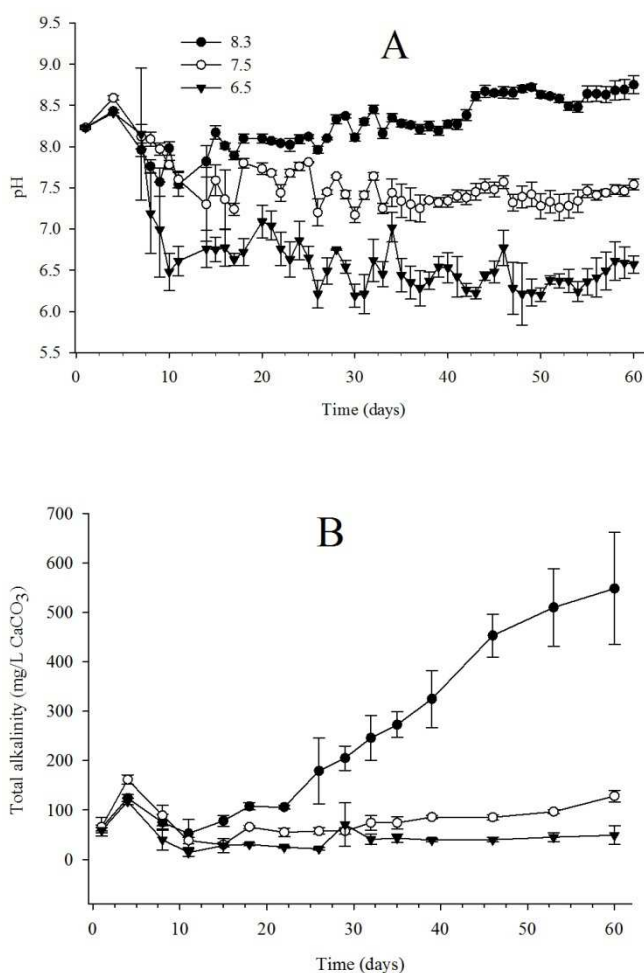


Fig. 1. pH (A) and total alkalinity (B) levels in biofloc technology during nursery of Nile tilapia *Oreochromis niloticus* at 6.5, 7.5 and 8.3 pH levels. Data are mean ± SD of three replicate tanks per sampling time.

TAN mean concentrations were similar among the treatments, and reached maximum values on the fourth day in all treatments; however, for pH 8.3 safe levels

were observed from the seventh day, while that for 7.5 and 6.5 this condition was registered from the eleventh and fifteenth day, respectively (Fig. 2A). Although for most of the trial period, the TAN (< 0.1 mg/L) values may be considered very low.

The medium concentrations of NO_2^- differ between 6.5 and the other treatments. For pH 8.3 the maximum value occurred on the seventh day, while for pH 7.5 and 6.5 the maximum values of NO_2^- occurred on fifteenth and twenty-first days, respectively (Fig. 2B). However, the NO_2^- change was abrupt and all treatments show irrelevant values on the twenty-fifth day (<0.03 mg/L).

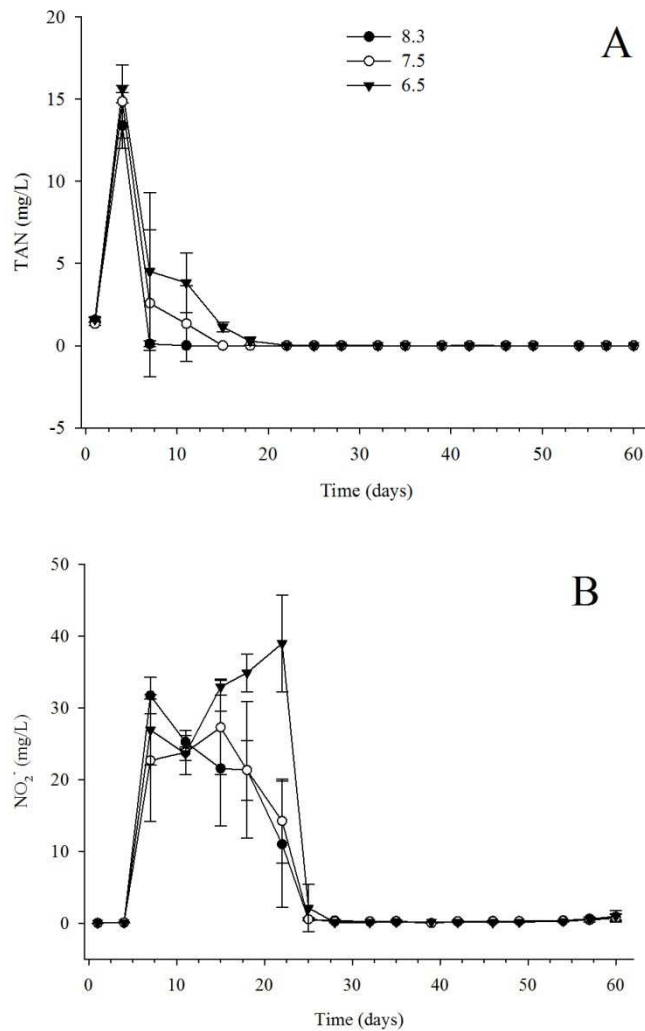


Fig. 2. Total ammonia (TAN) (A) and nitrite (NO_2^-) (B) in biofloc technology during nursery of tilapia *Oreochromis niloticus* raised at pH 8.3, 7.5 and 6.5. Data are mean \pm SD of three replicate tanks per sampling time.

The final means of TSS were similar among treatments throughout the experimental period (Fig. 3). However, the FV and FVI were superior for pH 6.5, compared to 8.3, while values at pH 7.5 were similar to the others.

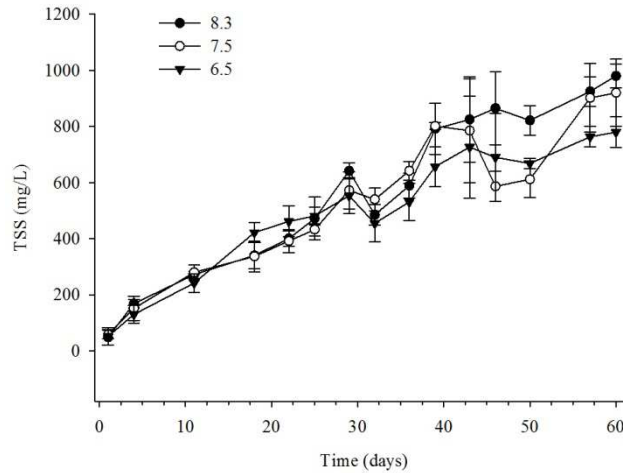


Fig. 3. Total solid suspension (TSS) in biofloc technology during nursery of Nile tilapia *Oreochromis niloticus* at pH 6.5, 7.5 and 8.3. Data are mean \pm SD of three replicate tanks per sampling time.

At final assay, NO_3^- and PO_4^- demonstrated similar values among the treatments at the final assay. The Ca^{+2} concentration was inferior for pH 8.3 compared to 6.5 and 7.5. While the Na^+ concentration did not differ among the treatments. Although, there was a positive relationship among the total alkalizing compound consumption and pH increase. But, the relative consumption of alkalizing compound was similar among pH 6.5 and 7.5, and inferior to pH 8.3

The results for growth, survival and food efficiency are presents in Table 3. To the utilize pH 7.5, the final weight and productivity were superior to those observed at pH 8.3, while the pH 6.5 results were similar to others. However, the final length, SGR, survival and FCR did not differ among the treatments.

The proximate composition of bioflocs evaluated at the final trial, demonstrates that pH 6.5 induces an increase in crude protein and ash content, compared to the other treatments (Tab. 4).

The hematologic evaluations of glucose, hematocrit and osmolality did not demonstrate significant differences (Tab. 5). Furthermore, the evaluations of ACAP

(Fig. 4A), LPO (Fig 4B), CAT (Fig 5A) and SOD (Fig 5B) showed no significant differences between treatments.

Table 3 Growth performance and feed utilization of Nile tilapia juveniles at different pH levels in biofloc system. Fishes (3.68 ± 0.93 g and 6.03 ± 0.50 cm) are stocked in 37.5 L tanks at 25 fish/tank, for 60 days.

	6.5	7.5	8.3
Final weight (g)	40.40 ± 4.10 ab	44.09 ± 0.93 a	37.10 ± 3.87 b
Final length (cm)	12.98 ± 0.58	13.16 ± 0.08	12.55 ± 0.61
DGR (g/day)	0.61 ± 0.06	0.67 ± 0.01	0.55 ± 0.06
Survival (%)	84.00 ± 4.00	80.00 ± 17.43	77.33 ± 6.11
FCR	1.08 ± 0.02	1.18 ± 0.07	1.06 ± 0.03
Net yield (kg/m³)	22.62 ± 2.30 ab	23.51 ± 0.49 a	19.12 ± 1.99 b

Each value represents mean \pm S.D (Tukey, $p < 0.05$). SGR – specific growth rate; FCR – food conversion rate.

Table 4 Crude protein (CP), crude lipid (CL) and ash (dry basis %) of biofloc maintained at different pH levels.

	6.5	7.5	8.3
CP	19.47 ± 0.74 a	16.76 ± 0.38 b	16.36 ± 0.74 b
CL	2.84 ± 0.75	2.11 ± 0.29	2.88 ± 0.17
Ash	47.22 ± 3.11 a	42.82 ± 3.25 b	39.68 ± 2.25 b

Each value represents mean \pm S.D (Tukey, $p < 0.05$).

Table 5 Hematologic analyses of Nile tilapia juveniles cultured at different pH levels on biofloc system.

	6.5	7.5	8.3
Glucose (mg/dL)	37.33 ± 5.20	39.5 ± 3.62	39.83 ± 2.92
Hematocrit (%)	30.2 ± 1.10	32.33 ± 1.13	29.66 ± 2.55
Osmolality (mOsm)	305.60 ± 12.63	316.66 ± 8.67	314.83 ± 9.12

Each value represents mean ± S.D (Tukey, $p < 0.05$; $n = 6$).

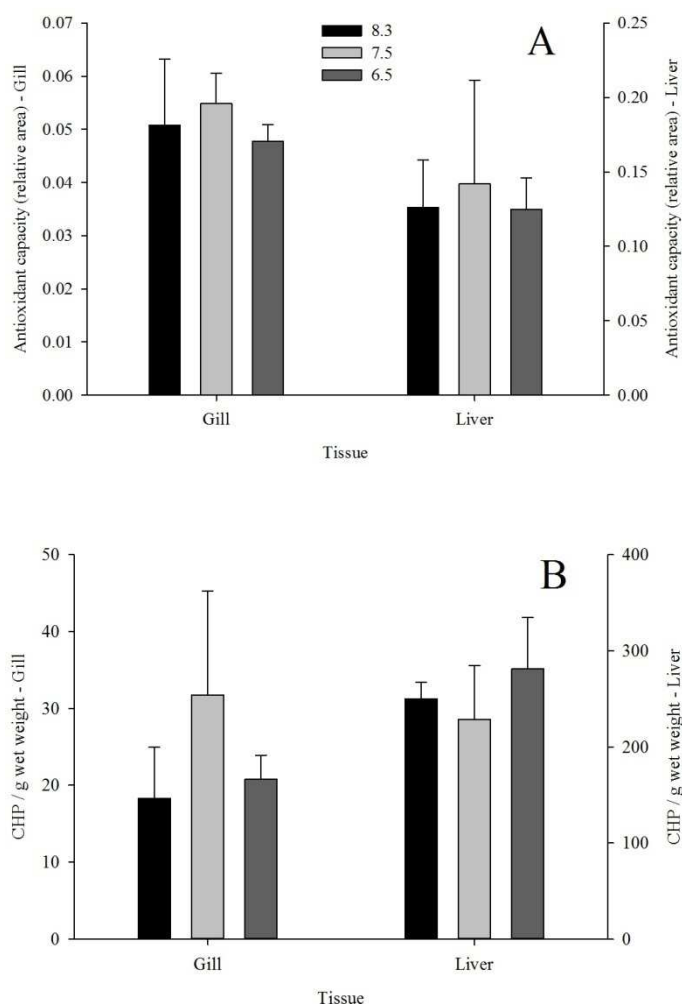


Fig. 4. Antioxidant capacity against peroxy radicals (ACAP, A) and Lipid Peroxidation (LPO, B) in gills and liver of Nile tilapia *Oreochromis niloticus* raised at pH 8.3, 7.5 and 6.5. (mean ± SD; ANOVA $p < 0.05$; $n = 6$).

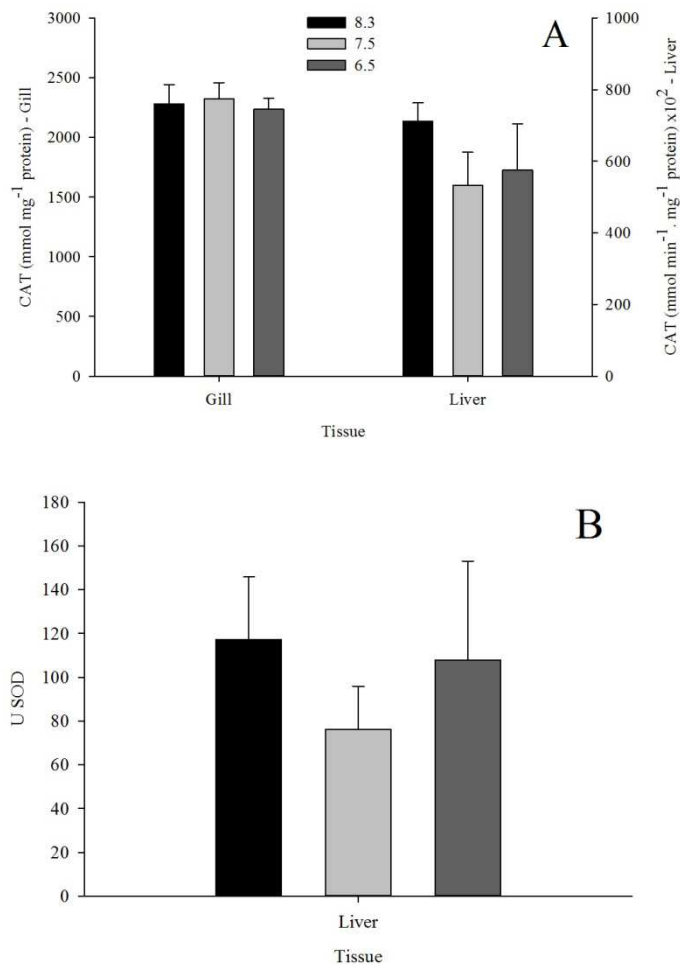


Fig. 5. Catalase (CAT) (A) and superoxide dismutase (SOD) (B) activity in gills and liver of Nile tilapia *Oreochromis niloticus* raised at pH 8.3, 7.5 and 6.5. (mean \pm SD; ANOVA $p < 0.05$; $n = 6$).

4. Discussion

Throughout the assay, the temperature and dissolved oxygen range remained at suitable levels for the survival and growth of Nile tilapia (Santos et al. 2013, Tran-Duy et al. 2012). According to the aim of this present study, the pH values differ among treatments, but the average of total alkalinity was equal for 6.5 and 7.5, and superior to 8.3.

Utilizing the fertilization with C organic strategy only for TAN > 1.0 mg/L is favorable to initial heterotrophic growth, which is responsible for rapid TAN consumption. Therefore, throughout the production cycle the C:N ratio decreases, which

is favorable to autotrophic growth. This strategy of maintenance of C:N ratio, proved to be efficient for nitrogenous cycling.

The pH influences the growth of both bacterial groups, and in general, may be considered the ideal range of pH for heterotrophic and autotrophic in the range from 7.0–10.0 (Mayo and Noik 1996) and 7.0–8.0 (Chen et al 2006), respectively. In the present study, the results demonstrate no influence of pH on the first step of nitrification, because the total oxidation of ammonia to nitrite was virtually equal among the treatments. However, for second step occur influences of pH, specifically related to nitrification velocity, being the maximum value reached to pH 6.5, followed by 7.5 and 8.3.

Several experiments were performed to demonstrate the relationship between pH and nitrification rate. For example, Jimenez et al. (2011) demonstrated that nitrification is high at pH 7.5, reduce at 7.0 and undetectable at 6.5. Tarre and Green (2004) evaluated reactor activity and obtained a high nitrification rate for water with pH 4.3. Apart pH, the alkalinity also affect the nitrification, and results featured for Chen et al. (2012) show the strong influence of C inorganic concentration, especially for the oxidation of ammonia to nitrite.

Our hypothesis, that nitrification would be compromised at pH 6.5, was not confirmed, because both TAN and NO_2^- levels remained low after the initial reduction. This may be explained by the diversity of organisms in inoculums and the conditions of water quality maintained throughout the assay, favoring the establishment of specific groups. Villano et al. (2010) demonstrate that conditions of pH are associated with the selection of microbial populations, favoring a strong response capacity to environmental changes, as well as the capacity to regulate nutrient absorption and metabolism (Lauro et al. 2009). Thus, the present study shows that it's possible to maintain nitrification at pH 6.5, through the selection or adaptation of microbial groups.

At the beginning of the experiment, mortalities in all treatments were recorded and attributed to the increase in ammonia and nitrite. These values were proximate to lethal concentration at 96 h of 0.96 (NH_3) (Evans et al. 2006) and 81 mg NO_2^-/L (Atwood et al. 2001). Interestingly, all treatments reached irrelevant levels after the twenty-fifth day, which remained true until the final assay, and no mortalities were recorded.

The low or zero water exchange characteristic of the BFT system, is associated with the correct management of water quality, providing for establishment and growth of nitrifying bacteria (Avnimelech 2012). This favors the accumulation of NO_3^- and phosphate. In the present study, the NO_3^- concentrations at ultimately reached levels that indicate intense nitrifying activity, but more studies about the nitrate toxicity for Nile tilapia are needed.

At the final assay, PO_4^- levels were low. Similarly, Luo et al. (2014) demonstrate low concentrations of phosphate on BFT compared to RAS, indicating PO_4^- cycling by microorganisms is present in bioflocs. Similarly, Ray and Lotz (2014) also found low concentration of PO_4^- , which they attributed to absorption by heterotrophic bacteria, as demonstrate for Longnecker et al. (2010). The monitoring of NO_3^- and PO_4^- is fundamental, both for reuse as well as for discharge as effluent.

At high stocking densities the TSS and their control are a limiting factor, and the removal of excessive bioflocs is necessary (Ray et al. 2010). After the fortieth day the TSS reached values superior to 600 mg/L in all treatments; however, no excessive reduction of dissolved oxygen or nitrification oscillation occurred. Although the sludge volume removed on each clarification was not recorded, increases in pH are accompanied by a larger number of clarifications compared to pH 6.5, indicating higher solid production in the system under higher pH values. Thus, keeping the BFT system at pH 6.5 can also be an advantage, related to less solid production.

The FV and TSS proportion represent the FVI, which is related to biofloc density (length, weight and porosity). According to De Schryver et al. (2008), the ideal range of FVI is between 40-60 mL/g for waste treatment systems, which is considered an intermediate range that favors the permanency in the water column as well as sedimentation. The present results demonstrate FV and FVI reduction when utilized pH 6.5 or 8.5, while the upper FV and FVI values were obtained for pH 7.5. The higher FVI (less density) reached can be attributed to further development of heterotrophic bacteria that give low density to bioflocs, due to the pH 7.5 is within the ideal range for growth of these group (Mayo and Noyk 1996), in contrast to the other treatments.

Management of the BFT system favors the ionic increase, because of the alkalinity correction and water reposition lost by evaporation and during clarification process. Recently, Pérez-Fuentes et al. (2016) have demonstrated increased dissolved

salts with increases in the C:N ratio. Besides that, NaHCO_3 utilization results in Na^+ accumulation, and the present study found that the final concentration of these ion did not differ among the treatments. However, greater use occurred for NaHCO_3 with increased pH. Most likely for a more long time, the Na^+ concentration is the largest for the highest pH levels.

Although total hardness did not differ among the treatments, the Ca^{+2} concentration demonstrated reduction at increases pH, and these can be attributed to higher production of extracellular polymeric substances (EPS) for bacteria and biofloc removal according to increases in pH. The Ca^{+2} adhesion in floc structures is associated with the neutralization of negative charges of EPS, favoring the adhesion of microorganisms (Wilén et al. 2008, Ye et al. 2016). The Na^+ and Ca^{+2} are fundamental to the formation of floc structure (Peeters et al. 2011), and their accumulation throughout the cycle deserves further investigation in the context of BFT system, with particular attention to water reuse.

The nutritional composition of bioflocs is a form to characterize the nutritional contribution to fishes. Our results demonstrate that crude protein remained below of the values previously reported in the literature, while crude lipids it is within the range previously demonstrated (Azim et al. 2007, Ekasari et al. 2010, Lopes-Elías and Moreno-Arias 2015). The pH 6.5 presented the highest values of crude protein and ash content, and these can be attributed to lower biofloc removal, pH values of 7.5 and 8.3 results in higher clarification, and this reduces nutrient content, as demonstrated by Ray et al. (2010).

Although when using pH 6.5, the NO_2^- values were superior during the initial part of the assay, there was no prejudice to growth and productivity, and these treatments present similar results to those obtained at pH 7.5. The Nile tilapia is tolerant to a wide range of pH (Lim and Webster 2006, El-Sherif et al. 2009), but our results demonstrate growth reduction for pH 8.3. Similar results were obtained for Rebouças et al. (2015), who evaluated juveniles Nile tilapia growth in ponds. The authors demonstrated best growth when fish were maintained at pH 4-6, as compared to pH 8.0. In other perspective, Cavalcante et al. (2012) demonstrated improvement in Nile tilapia growth when hardness:alkalinity ratio was maintained equal or superior to 1.0, which in the present study was achieved at pH 6.5 and 7.5.

The excessive increase in alkalinity at 8.3 treatments can be attributed to the limiting of HCO_3^- on pH elevation, and this could influence growth. The accumulation of HCO_3^- in water may have reduced the excretion of these ions and Cl^- absorption. Alternative ways of ionic regulation may have been used, mainly resulting in higher energy expenditure with osmoregulation, related to Na^+K^+ -ATPase higher activity (Boeuf and Payan 2001), since the osmolality and hematocrit remained equal between treatments. Hematologic and oxidative parameters can be used to demonstrate the health status of fishes. Our results do not demonstrate alteration when evaluating the hematologic parameters of glucose, hematocrit and osmolality, which showed no alteration. These may indicate that system conditions remained appropriate among the treatments, although growth was reduced for pH 8.3.

In relation to oxidative stress, the evaluation of CAT and SOD resulted in no difference. The SOD is responsible for the conversion of superoxide anions to the less toxic product H_2O_2 and CAT catalyses the H_2O_2 removal, producing H_2O and O_2 . (Storey 1996).

Similarly, the ACAP and LPO showed no difference among treatments. This may be attributed to pH range tested, which is suitable for the Nile tilapia. Oxidative stress has been associated with inadequate system conditions in aquaculture, leading to alterations in SOD and CAT activity after exposure to ammonia exposure (Sinha et al. 2014), cellular damage related to alkaline challenges (Yao et al. 2012) and increased on ROS levels for Nile tilapia at pH 5.3 (Mai et al. 2010). For this reason, the absence in alteration in oxidative stress parameter observed in the present study would indicate that the health status of organisms is good.

5. Conclusion

Initiating the biofloc formation with alkaline water could be advantageous for nitrifying bacteria growth, resulting in the oxidation of nitrogenous forms faster than in water with pH below 8.0. However, the elevated consumption for nitrification and consequent pH reduction represents no damage to bioflocs, with nitrogenous oxidation maintained even at pH 6.5. Furthermore, at pH 6.5, the biofloc density is higher (lower FVI) and TSS production is reduced, resulting in less biofloc removal.

The best results for final weight and net yield were observed for pH 7.5 and 6.5. Evaluations of the biochemical/physiological parameters showed no difference among the treatments, proving that the conditions of pH 6.5–8.3 are not challenging to fish. Thus, the utilization of pH 7.5–6.5 could be represents economic advantages, related to reduced TSS production and less alkalizing compound consumption in the BFT system.

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CAPÍTULO III

Concentração de dureza da água durante berçário de tilápia do Nilo *Oreochromis niloticus* (L.) em sistema de bioflocos

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Concentrações de dureza da água durante berçário de tilápia do Nilo *Oreochromis niloticus* (L.) em sistema de bioflocos

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Resumo

A produção de tilápia do Nilo utilizando a tecnologia dos bioflocos (BFT) é uma das formas mais sustentáveis de produção, devido à ciclagem dos nitrogenados inorgânicos pelos microorganismos. Entretanto, com a intensificação e a baixa renovação de água, ocorre significativo consumo de alcalinidade, tornando necessária a utilização de alcalinizante, como o hidróxido de cálcio – $\text{Ca}(\text{OH})_2$, que favorece o acúmulo de cálcio ao longo do cultivo. Nesse contexto, foi realizado o presente experimento de 20 dias de duração, para investigar o aumento da dureza total avaliando: qualidade da água, crescimento, sobrevivência e eficiência alimentar de juvenis de tilápia em sistema BFT. Foram distribuídos 30 peixes ($1,51 \pm 0,38$ g) por unidade experimental (vol. útil 37,5 L), testando em triplicata o controle (sem correção), 250 e 700 mg L^{-1} CaCO_3 de dureza total, utilizando CaCl_2 . No início do experimento, ocorreu elevação da amônia total (TAN) de forma similar para todos os tratamentos, entretanto, rapidamente atingiu níveis seguros. Enquanto que a oxidação de nitrito à nitrato (NO_3^-) foi um pouco mais lenta para o tratamento 700, embora rapidamente todos tenham atingido níveis irrisórios. O NO_3^- apresentou menor concentração final para o controle, e isso demonstra maior absorção de N inorgânico pelas bactérias heterotróficas quando

comparado aos demais tratamentos. Enquanto os sólidos suspensos totais (SST) apresentaram relação positiva com a dureza, e isso se deve à maior adesão de cálcio na estrutura dos bioflocos, aumentando a densidade, e tornando o índice de volume de floco (FVI) nos tratamentos com correção da dureza mais estável ao longo do ensaio. Ainda mais, o peso final dos peixes nos tratamentos 250 ($7,57 \pm 0,40$ g) e 700 ($7,37 \pm 0,35$ g) foram superiores ao controle ($6,30 \pm 0,11$ g). Enquanto a sobrevivência (aprox. 80%) e a taxa de conversão alimentar (aprox. 1,0) não apresentaram diferença entre os tratamentos, porém apresentam valores satisfatórios e indicam a sustentabilidade do sistema. Finalmente, as análises da composição iônica da água demonstram as concentrações iniciais e finais de Ca^{+2} , Na^{+} e Cl^{-} , e no presente estudo não comprometeram os bioflocos ou o crescimento dos peixes, entretanto, a longo prazo fica evidente a importância do monitoramento desses íons em sistema com baixa renovação de água, como o BFT.

Palavras chave: cálcio, sódio, cloreto, BFT, bactérias nitrificantes

Introdução

Existe uma crescente busca mundial em utilizar de forma sustentável os recursos naturais, e em aquicultura o desafio é o desenvolvimento de sistemas intensivos que utilizem menos água, produzam menos efluentes e alcancem o melhor aproveitamento de nutrientes. Utilizando baixa ou nula renovação de água, a *tecnologia dos bioflocos* (BFT) tem sido demonstrada como uma importante alternativa para suprir essas necessidades (Crab, Defoirdt, Bossier & Verstraete 2012).

O sistema BFT caracteriza-se por possuir agregados microbianos (bioflocos) que absorvem o nitrogênio inorgânico dissolvido (De Schryver, Crab, Defoirdt, Boon & Verstraete 2008). Esses bioflocos permanecem em suspensão na água e são consumidos pelas tilápias do Nilo, portanto, são considerados como suplementos nutricionais (Avnimelech 2009; Ekasari, Angela, Waluyo, Bachtiar, Surawidjaja, Bossier & De Schryver 2014).

A ciclagem do N inorgânico dissolvido em BFT, ocorre principalmente pelas bactérias pertencentes aos grupos: heterotrófico e quimioautotrófico (autotrófico).

Inicialmente, o sistema BFT foi desenvolvido baseado no crescimento das heterotróficas, realizando fertilizações com carbono (C) orgânico (relação 15:1 C:N) (Avnimelech 1999). Esse grupo possui como principais características a alta taxa de crescimento e capacidade de rápida absorção de nitrogênio amoniacal (Ebeling, Timmons & Bisogni 2006).

Entretanto, tem sido demonstrado que as estratégias de manutenção do sistema BFT, utilizando relações C:N mais baixas, seja pela fertilização com C orgânico ou pela ração, podem favorecer o sistema a manter comunidades autotróficas (Ebeling et al. 2006, Avnimelech 2012, Luo, Avnimelech, Pan & Tan 2013a). A utilização desse grupo, responsável pela nitrificação, pode representar algumas vantagens, como a redução na produção de sólidos suspensos e menor consumo de O₂ pelos bioflocos (Ray & Lotz 2014). Entretanto, as autotróficas consomem o dobro de alcalinidade que as heterotróficas (7,0 versus 3,5 g Alc. g⁻¹ N⁻¹), favorecendo a redução do pH do sistema (Ebeling et al. 2006).

Consequentemente, em sistema BFT é necessária a adição permanente de compostos alcalinizantes, e uma das alternativas utilizadas é o hidróxido de cálcio, também denominado cal hidratada (Ca(OH)₂) (Furtado, Gaona, Poersch & Wasielesky 2014). Ao se diluir em água, produz a seguinte reação de neutralização: $\text{Ca(OH)}_2 + 2\text{H}^+ \rightarrow \text{Ca}^{+2} + 2\text{H}_2\text{O}$. Trata-se de uma alternativa barata, que possui rápida diluição e é eficaz na correção da alcalinidade e do pH. Entretanto, a utilização de Ca(OH)₂ durante o cultivo em sistema BFT, associada às reposições de água devido às clarificações e à evaporação, favorece o acúmulo de Ca⁺² ao longo do cultivo, tornando a água excessivamente dura.

A dureza total representa a soma dos cátions divalentes, principalmente Ca⁺² e Mg⁺², e é expressa em mg L⁻¹ de CaCO₃ (Boyd, Tucker & Someidhivej 2016). O Ca⁺² possui importante função relacionada à floculação e adesão dos bioflocos, devido ao *cation bridging theory* (Sobeck & Higgins 2002). Foi demonstrado por Luo, Liang, Tan, Yao, Zhang & Lu (2013b) que ao aumentar a concentração de Ca⁺² em bioreatores, ocorre proporcional redução no tamanho dos bioflocos, maior força de adesão e capacidade de sedimentação, entretanto, há redução no teor de proteína e na capacidade de absorver nitrogênio.

No aspecto fisiológico, os teleósteos absorvem o Ca^{+2} do meio principalmente pelas brânquias, e quando em concentrações muito baixas, a principal fonte é a partir da alimentação (Baldisserotto 2011). O Ca^{+2} é importante na estabilização das *tight junctions*, resultando em menor perda iônica pelas brânquias (Wood 2001). Desta forma, a elevação da dureza é uma estratégia utilizada quando em baixo pH, reduzindo o desafio osmorregulatório (Parra & Baldisserotto 2007). O excesso ou déficit de cálcio no meio pode ser prejudicial ao crescimento dos peixes, e ao avaliar o crescimento de tilápias do Nilo, Cavalcante, Silva, Pinheiro, Akao & Sá (2012) demonstraram melhor desempenho quando a dureza total é $> 20 \text{ mg CaCO}_3 \text{ L}^{-1}$, ainda mais, os peixes obtiveram crescimento superior quando mantida a relação dureza:alcalinidade entre 1,0 e 3,0.

Portanto, em BFT há uma forte interação entre a composição dos bioflocos e os parâmetros de qualidade da água, como alcalinidade, pH e dureza, e o conjunto desses fatores afeta diretamente o desempenho dos peixes. Os efeitos dos níveis de Ca^{+2} não são explorados em sistema BFT para tilápias do Nilo, e a maioria dos estudos tem sido desenvolvidos em sistemas de tratamento de efluentes com lodo ativado (Peeters, Dewil, Lechat & Smets 2011; Ye, Yang, Zhao & Ren 2016) e em *sequence batch reactor* (SBR) utilizando BFT (Luo et al. 2013a). Nesse contexto, este estudo investiga os níveis de dureza total da água avaliando a sobrevivência, o crescimento e a qualidade da água de tilápias do Nilo durante berçário em sistema BFT.

Material e métodos

Peixes e desenho experimental

Os juvenis de tilápia do Nilo monossexo (Genética Premium) foram adquiridos da piscicultura comercial Aquabel (Rolândia, PR, BR) e transportados ao Laboratório de Piscicultura do Chasqueiro (Universidade Federal de Pelotas, Rio Grande do Sul, Brasil) onde ocorreu o experimento.

Para aclimação, os peixes foram mantidos em sistema de recirculação por 7 dias até o início do experimento. Durante a aclimação e o experimento, as tilápias foram alimentadas com ração comercial (1mm, 45% Proteína Bruta, Pirá Mirim, Guabi Nutrição e Saúde Animal, BR) distribuída as 07:30, 10:30, 13:30, 16:30 e 19:30 h. A

taxa de arraçoamento utilizada para peixes de 1,5 – 3,0 g foi 10% (peso úmido/dia), enquanto que a partir de 3 g foi 8%. Durante o ensaio, foram realizadas biometrias semanais (n = 10) para ajuste da quantidade de ração.

O experimento teve duração de 20 dias, e foram distribuídos 30 peixes por unidade experimental (vol. útil 37,5 L) com peso inicial de $1,51 \pm 0,38$ g. As caixas possuíam duas pedras de ar ao fundo para aeração contínua e aquecedor (100W) com termostato (regulado para 28°C). Flocos microbianos previamente produzidos com tilápias do Nilo foram inoculados no sistema, a uma taxa de 10% do volume total de água.

Os tratamentos testados foram controle (sem correção), 250 e 700 mg $\text{CaCO}_3 \text{ L}^{-1}$ de dureza total utilizando cloreto de cálcio – CaCl_2 (P.A. Synth, BR), todos realizados em triplicata e para elevar a dureza. A concentração de CaCl_2 utilizada para os tratamentos foi de 0,15 e 0,44 g L^{-1} .

Quando a amônia total (TAN) atingiu valor igual ou superior a 1.0 mg L^{-1} , foi realizada a adição de melado de cana de açúcar ($\approx 45\%$ C) para a correção da relação entre C:N (15:1), conforme demonstrado por Avnimelech (1999). Para manter o nível de sólidos suspensos totais (SST) entre 400-600 mg L^{-1} , foram realizadas remoções dos sólidos pela sedimentação de 25% do volume da caixa em um balde por 1,0 h, após esse período o sobrenadante foi devolvido ao tanque e o sedimentado (*sludge*) foi mensurado. Durante o ensaio, foi realizada clarificação apenas uma vez em duas réplicas do tratamento 700 mg $\text{CaCO}_3 \text{ L}^{-1}$, e por esse motivo não foi aplicado teste estatístico para essa variável. O valor de *sludge removed* (SR) foi calculado para ser expresso como SR (L m^{-3}).

Para a correção da alcalinidade e do pH foi utilizado o bicarbonato de sódio – NaHCO_3 (P.A. Synth, BR), sendo calculada a quantidade de acordo com os valores de pH diários, e adicionado diariamente após a última alimentação. Ainda, durante o experimento não houve renovação de água, e semanalmente o volume de água foi reajustado devido às perdas com as clarificações e evaporação.

Ao final do ensaio foi quantificado a ração total e o composto alcalinizante total utilizado, sendo calculado o percentual de composto alcalinizante utilizado em relação à ração (AC%).

Qualidade da água e desempenho zootécnico

A temperatura e o oxigênio foram medidos duas vezes ao dia (07:15 e 15:30 h), utilizando YSI PRO 20 (YSI Incorporated, Yellow Springs, OH, USA). O pH foi medido diariamente (17:00 h) com pHmetro digital de bancada (HI 2212, HANNA Instrument, Woonsocket, RI, USA).

Para TAN e NO_2^- as amostras de água foram coletadas antes da primeira alimentação do dia, enquanto que para o SST, FV, alcalinidade, dureza as amostras foram coletadas as 13:15 h. Duas vezes por semana, foram dosados a amônia total (TAN) (UNESCO 1983), o nitrito e a alcalinidade total (APHA 1998), os sólidos suspensos totais (SST) (Strickland & Parsons 1972), o volume de sólidos suspensos (FV; Cone de Imhoff - 30 min) e a dureza total (Eaton et al. 2005). O índice de volume de floco foi determinado pela seguinte fórmula: $\text{FVI} = \text{FV (mL)} / \text{TSS (g)}$ (Yousuf 2013).

O NO_3^- e o PO_4^- (Aminot & Chaussepied 1983) o Na^+ (fotômetro de chama, B462, Micronal, BR), o Ca^{+2} (cálcio arsenazo, Doles, BR) e o Cl^- (tiocianato de mercúrio, Vida Biotecnologia, BR) foram dosados apenas no primeiro e último dias do experimento.

Ao final do ensaio, foram quantificados e pesados todos os peixes para avaliação dos seguintes índices de sobrevivência, crescimento e conversão alimentar:

- a) Taxa de crescimento diário (DGR; g dia^{-1}) = aumento da biomassa de peixes (g) / tempo (dias)
- b) Taxa de sobrevivência (%) = $100 (\text{Nf}-\text{Ni}) / \text{Ni}$
- c) Produtividade (kg m^{-3}) = aumento da biomassa de peixes (g) *1000 / Vol (L)
- d) Taxa de conversão alimentar (FCA) = quantidade de ração (g) / aumento da biomassa de peixes (g)

Onde Ni e Nf representam o número de peixes inicial e final, respectivamente.

Análises estatísticas

Inicialmente os dados foram testados para normalidade (Shapiro-Wilk) e homocedasticidade. Após, foram comparados por ANOVA (One way), e quando houve diferença foi realizado Teste de Tukey ($p < 0,05$). As análises foram realizadas no software SigmaPlot 12.0 (Systat Software, Inc., Chicago, IL). Todos os dados são demonstrados como Média \pm Desvio Padrão.

Resultados e discussão

Os resultados de qualidade da água são demonstrados na Tabela 1. O intervalo de temperatura do presente estudo se manteve entre 26-30°C, o que é considerado ideal para a sobrevivência e crescimento de tilápia do Nilo (Santos, Mareco & Silva 2013). Enquanto que o intervalo de oxigênio dissolvido foi de 4-7 mg L⁻¹, o ideal para manter a sobrevivência e o crescimento (Tran-Duy, Dam & Schrama 2012).

O pH durante o ensaio demonstrou considerável oscilação (8,3 a 6,6) (Fig. 1), sendo o valor médio do controle igual ao tratamento 250 e superior ao 700. Entretanto, as médias de pH estão dentro do intervalo considerado ideal para a sobrevivência de tilápias do Nilo (Rebouças, Lima, Cavalcante & Sá 2015). Essa pequena diferença no pH deve ter ocorrido porque a utilização de NaHCO₃ em água com alta dureza favorece a seguinte reação: $\text{Ca}^{+2} + 2\text{HCO}_3^- \rightarrow \text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O}$. Portanto, a presença de Ca^{+2} limita o aumento do pH, devido a precipitação do CO_3^{-2} como CaCO₃ (Boyd et al. 2016). Entretanto, no presente estudo a diferença de pH entre os tratamentos pode ser considerada pequena, pois a alcalinidade média não diferiu entre os tratamentos.

As águas podem ser classificadas de acordo com os valores de dureza, e para o controle, é considerada moderadamente dura, enquanto que para os tratamentos 250 e 700 é classificada como dura e muito dura, respectivamente (Boyd 2015). Como parâmetro, o tratamento 700 apresentou concentração de Ca^{+2} próximo a água do mar (400 mg L⁻¹) (Boyd 2015). Embora a dureza total seja representada pela soma dos cátions divalentes, o Ca^{+2} é o que possui maior afinidade de ligação nos bioflocos (Nguyen, Hankins & Hilal, 2007; Luo et al. 2013b), por isso o Mg^{+2} não foi considerado neste estudo.

Tabela 1. Qualidade da água durante berçário de tilápia do Nilo *Oreochromis niloticus* em sistema BFT utilizando níveis de dureza da água: controle, 250 e 700 mg/L CaCO₃.

	Controle	250	700
Temperature (°C)	28,50 ± 0,03 (26,7 – 30,5)	28,54 ± 0,03 (27,1 – 30,5)	28,42 ± 0,11 (26,7 – 30,3)
O₂ (mg/L)	6,14 ± 0,30 (5,68 – 7,15)	6,03 ± 0,53 (4,15 – 7,05)	6,12 ± 0,31 (5,52 – 7,06)
pH	7,38 ± 0,40 a	7,28 ± 0,35 ab	7,21 ± 0,38 b
Alcalinidade total (mg CaCO₃ L⁻¹)	37,83 ± 3,75	42,50 ± 2,17	40,00 ± 2,64
Total hardness (mg CaCO₃ L⁻¹)	78,66 ± 0,66 a	277,11 ± 4,01 b	710,22 ± 11,00 c
TAN (mg L⁻¹)	1,01 ± 1,34	1,05 ± 1,54	1,76 ± 1,74
NO₂ (mg L⁻¹)	4,78 ± 5,75	8,40 ± 9,74	9,69 ± 11,15
NO₃⁻ (mg L⁻¹)	Inicial	1,33 ± 0,57	2,00 ± 1,00
	Final	36,33 ± 4,61 a	46,06 ± 0,11 b
TSS (mg L⁻¹)	237,66 ± 10,75 a	297,66 ± 8,69 b	351,83 ± 13,83 c
FV (ml L⁻¹)	22,75 ± 9,36	29,5 ± 3,84	15,37 ± 0,54
FVI (mL g⁻¹)	87,16 ± 31,06 a	82,16 ± 8,86 ab	36,29 ± 3,88 b
BR (L m⁻³)	-	-	8,13 ± 0,94
PO₄⁻ (mg L⁻¹)	Inicial	1,68 ± 0,83	2,5 ± 1,41
	Final	12,33 ± 1,52	10,93 ± 1,10
Total AC (g)	12,66 ± 1,15 a	17,66 ± 0,57 b	20,33 ± 1,52 b
Relativo AC (%)	12,05 ± 0,87 a	13,22 ± 0,14 a	15,45 ± 1,23 b

Dados representam Média ± D.P. (Tukey, $p < 0.05$). Entre parêntesis os valores máximo e mínimo. TAN – amônia total, TSS – sólidos suspensos totais, FV – volume de floco, FVI – índice do volume de floco, SR – biofloco removido, AC – consumo de alcalinizante.

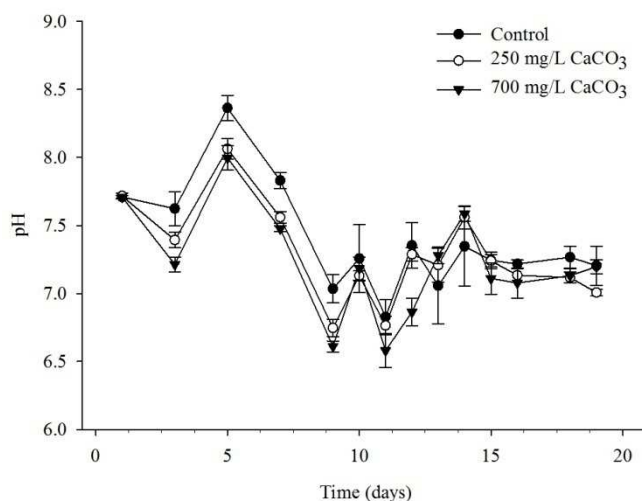


Fig 1. Valores de pH em sistema de bioflocos durante berçário de tilápia *Oreochromis niloticus* mantidos aos níveis de dureza total de controle, 250 e 700 mg L⁻¹ CaCO₃. Os dados representam média ± DP, de três réplicas por tempo amostral.

Conseqüentemente, o aumento dos níveis de Ca⁺² tem implicações à estrutura e composição dos bioflocos, como: maior densidade (menor FVI), maior força de adesão e rigidez estrutural (Ren, Liu, Sheng, Liu, Yu, Zhang & Zhu 2008), menor tamanho e alteração do conteúdo protéico e conseqüentemente menor capacidade de absorver nitrogênio inorgânico (Luo et al. 2013b, Ekasari et al. 2014). Em relação à TAN, durante o experimento não houve diferença entre os tratamentos (Fig. 2 A). Entretanto, a segunda etapa da nitrificação, a conversão do NO₂⁻ à NO₃⁻, demonstrou ser um pouco mais lenta inicialmente (Fig 2 B) para os tratamentos com elevação da dureza.

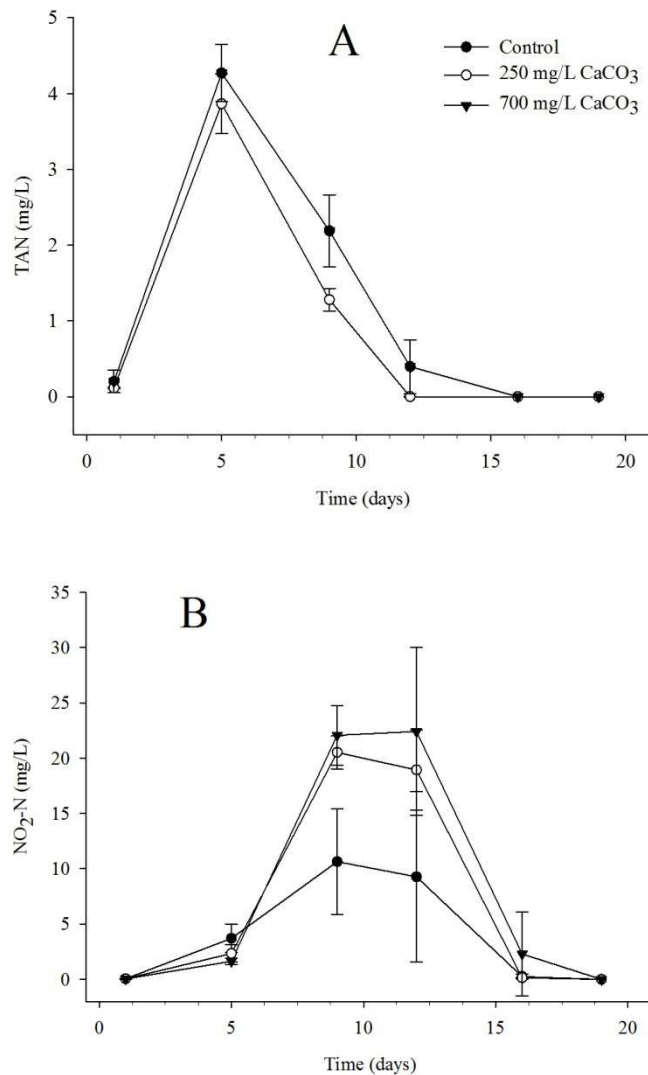


Fig. 2. Amônia total (TAN) e nitrito (NO_2^-) em sistema de bioflocos durante berçário de tilápia do Nilo *Oreochromis niloticus* mantidos aos níveis de dureza total de controle, 250 e 700 mg L^{-1} CaCO_3 . Os dados representam média \pm DP, de três réplicas por tempo amostral.

A redução na velocidade de oxidação de nitrito à nitrato pode ser atribuída a dois fatores: (1) a redução no tamanho dos bioflocos e (2) alteração da composição bacteriana presente nos bioflocos. Como citado anteriormente, bioflocos menores apresentam redução no conteúdo protéico, indicando menor capacidade em absorver nitrogênio inorgânico (Ekasari et al. 2014, Luo et al. 2013b). Além disso, em sistemas de tratamento de efluentes, tem sido demonstrado que concentrações maiores que 150 mg L^{-1} de Ca^{+2} , alteram a composição microbiana dos flocos (Ye et al. 2016). Conseqüentemente, no período de formação dos bioflocos há uma oscilação no sistema,

devido ao processo de seleção e crescimento das bactérias, que pode ser demorado, causando redução temporária na taxa de nitrificação.

Devido à utilização de sistema contendo bactérias autotróficas, ao final do experimento ocorreu acúmulo de NO_3^- , e um tema que deve ser mais explorado é a fertilização para estimular a absorção de nitrato em BFT, como citado por Schneider, Sereti, Eding & Verreth (2007) e Luo et al.(2013b). Nossos resultados demonstram que a menor concentração de NO_3^- para o controle pode indicar a maior assimilação de TAN pelas bactérias heterotróficas, comparado aos tratamentos com correção da dureza. Esse resultado é interessante, pois sugere inibição das heterotróficas ao aumentar o nível de Ca^{+2} .

Além disso, houve acúmulo de PO_4^- em todos os tratamentos. É bem estabelecido que a elevação do Ca^{+2} dissolvido pode favorecer a formação de fosfato de cálcio, que precipita no sistema (Li, Xu, Parise & Phillips, 2012). Ainda mais, Schneider, Sereti, Eding & Verreth (2006) demonstraram que ao manter a relação 15:1 (C:N) há favorecimento de absorção de fósforo pelas bactérias heterotróficas. Entretanto, no presente estudo talvez não tenha ocorrido nenhuma das vias de consumo do fósforo devido ao curto período experimental e a baixa relação C:N utilizada.

Em sistema BFT, o reuso de água associado à grande quantidade de ração fornecida, favorece o acúmulo de TSS. Para a produção do camarão marinho *Litopennaeus vannamei* em BFT, tem sido recomendada a manutenção dos TSS entre 400-600 mg L^{-1} (Ray, Lewis, Browdy & Leffler 2010; Schweitzer, Arantes, Costódio, Espírito Santo, Arana, Seiffert & Andreatta 2013). As médias de TSS do presente estudo permaneceram a níveis que podem ser considerados adequados, embora tenham demonstrado relação positiva com o aumento da dureza (Fig. 3 A). Isso ocasionou a clarificação de duas réplicas do tratamento 700, resultando em remoção de aprox. 8 L m^{-3} de bioflocos para esse tratamento.

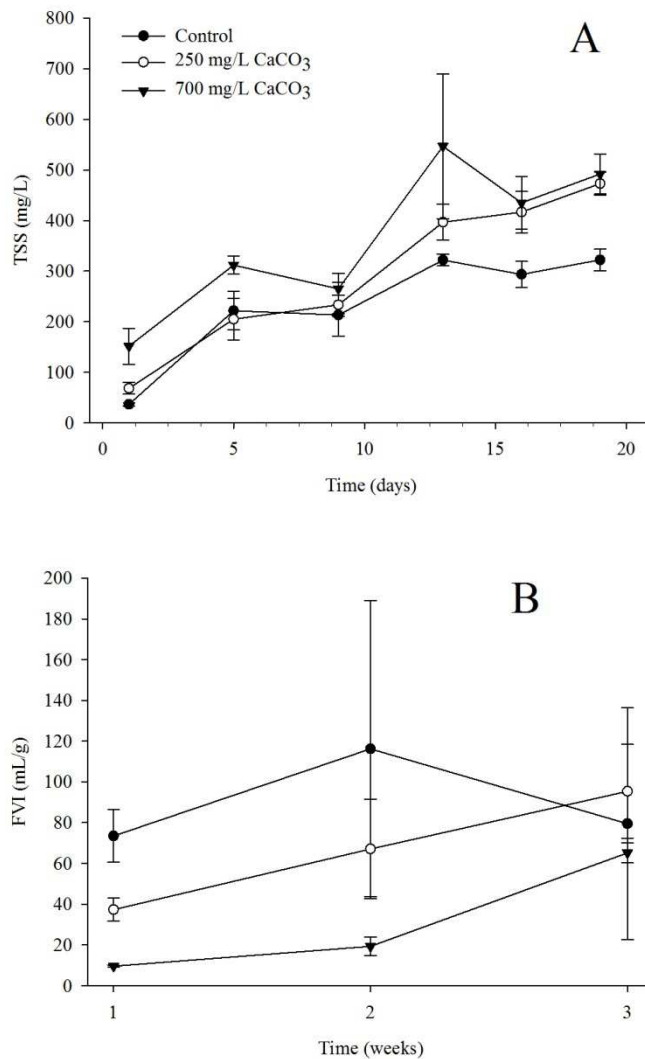


Fig. 3. Sólidos suspensos totais (TSS) (A) e Índice de volume de floco (FVI) (B) em sistema de bioflocos durante berçário de tilápia do Nilo *Oreochromis niloticus* mantidos aos níveis de dureza total de controle, 250 e 700 mg L⁻¹ CaCO₃. Os dados representam média ± DP, de três réplicas por tempo amostral.

A elevação dos TSS com o aumento da dureza pode ser relacionado à maior adesão de Ca⁺² à *extra-cellular polymer substances* (EPS) produzida pelas bactérias, conferindo maior peso e maior densidade aos bioflocos. Esses resultados são importantes, pois definir as clarificações de acordo com os valores de TSS para o *aquaculture manager* representa custo extra de manutenção, além da geração de resíduos.

A utilização de Ca⁺² tem sido comumente utilizada em sistemas de tratamento de efluentes que utilizam lodo ativado (*actived sludge*), principalmente devido à

capacidade de aumentar a coagulação-floculação e a sedimentação (Nguyen, Hilal, Hankins & Novak, 2008). No presente estudo, a capacidade de sedimentação é representada pelo FVI (Fig 3 B), que apresentou redução (maior densidade) quando utilizado $700 \text{ mg L}^{-1} \text{ CaCO}_3$ de dureza. Para o FVI, tem sido considerado ideal o intervalo entre $40\text{-}60 \text{ mL g}^{-1}$ (De Schryver et al. 2008).

Na segunda e terceira semanas, foram verificados aumentos excessivos do FVI em duas réplicas do tratamento controle, atingindo FVI de aprox. 180 mL g^{-1} (densidade extremamente baixa). Em sistemas de tratamento, esse resultado é tratado como o *bulking*, e tem sido atribuído ao intenso crescimento de microorganismos filamentosos e alta produção de *EPS*, o que confere à estrutura dos bioflocos alta porosidade, baixa densidade e capacidade de sedimentação (Hu, Qi, An, Xu, Zhang, Bai, Bao, Wen, Gu & Yang 2013). Em nosso laboratório, tem sido analisado que valores de FVI próximos a 200 mL g^{-1} (bioflocos com coloração clara e Cone Imhoff $> 100 \text{ mL}$) causam entupimento branquial, e rapidamente causa grande mortalidade de peixes. Liao, Droppo, Leppard & Liss (2006) demonstraram ser possível evitar o *bulking* ao reduzir o tamanho dos flocos, assim, nossos resultados também demonstram que utilizar o Ca^{+2} para elevar a densidade e reduzir os tamanhos dos bioflocos, é uma alternativa para prevenir o redução excessiva da densidade (aumento do FVI) em BFT.

Como já citado anteriormente, o intenso consumo de alcalinidade pelas bactérias ocasiona redução no pH, e a utilização de compostos alcalinizantes resulta em mudança na composição iônica da água. A tabela 2 apresenta a concentração de Ca^{+2} , Na^{+} e Cl^{-} da água no início e fim do ensaio. Obviamente, os níveis de Ca^{+2} e Cl^{-} aumentaram de acordo com o tratamento testado. Enquanto o Na^{+} aumentou significativamente ao longo do experimento, entretanto, assim como o Cl^{-} , a concentração final foi baixa e possivelmente não representou desafio osmorregulatório às tilápias.

Table 2. Composição iônica (mg L⁻¹) da água de sistema de bioflocos durante berçário de tilápia do Nilo *Oreochromis niloticus* utilizando correção da dureza total (controle, 250 e 700 mg L⁻¹ CaCO₃).

	Ca ⁺²		Na ⁺		Cl ⁻	
	Inicial	Final	Inicial	Final	Inicial	Final
Controle	60,40 ± 10,82 a	77,48 ± 20,40 a	11,40 ± 0,23	56,26 ± 6,42	1,10 ± 0,03 a	1,10 ± 0,01 a
250	98,62 ± 18,47 b	145,02 ± 4,62 b	12,88 ± 1,85	58,47 ± 0,80	1,33 ± 0,05 b	1,37 ± 0,02 ab
700	241,16 ± 9,61 c	295,97 ± 8,93 c	13,56 ± 3,53	59,41 ± 0,23	1,76 ± 0,02 c	2,59 ± 1,13 b

Letras diferentes representam diferença somente entre os tratamentos para o mesmo tempo. Os dados representam Média ± D.P. (ANOVA, Tukey, $p < 0,05$, $n = 3$).

Em sistemas com baixa renovação de água, o monitoramento das concentrações iônicas é fundamental, pois o reuso da água pode tornar a água salobra, principalmente devido aos íons provenientes dos compostos alcalinizantes. Estudos anteriores demonstraram que há uma melhora no crescimento de tilápias do Nilo quando utilizada água salobra (6-8‰) (Qiang, Wang, Kpundeh, He & Xu 2013). Entretanto, é possível que o desequilíbrio iônico (aumento excessivo de um único íon, como por ex. o Na⁺) por um longo período de cultivo possa comprometer o crescimento dos peixes, devido ao gasto energético com a osmorregulação (Boeuf & Payan 2001). Em outra via, em sistema BFT ao aumentar a salinidade do meio, há redução na composição nutricional dos bioflocos, como reportado previamente por Maicá, Borda & Wasielesky (2012), podendo prejudicar a absorção de N inorgânico pelos bioflocos.

Embora tenha ocorrido elevação do NO₂⁻ ao aumentar a dureza, a sobrevivência não diferiu entre os tratamentos e permaneceu a níveis aceitáveis. A toxicidade do NO₂⁻ é significativamente reduzida ao aumentar a concentração de Cl⁻ no meio, devido à competição de ambos os íons pela troca com o HCO₃⁻ (Atwood, Fontenot, Tomasso & Isely, 2001; Svobodova, Machova, Drastichova, Groch, Luskova, Poleszczuk, Velisek & Kroupova 2005). Entretanto, esse efeito não foi evidente no presente estudo, pois não

houve redução na mortalidade para os tratamentos que utilizaram CaCl_2 . Isso deve ter ocorrido principalmente porque os níveis de NO_2^- permaneceram elevados por um curto período de tempo e foram muito abaixo da CL_{50} – 96 h de 81 mg L^{-1} para juvenis de tilápia do Nilo (Atwood et al. 2001).

Em relação ao crescimento (Fig. 4), os tratamentos com elevação da dureza obtiveram maior peso final, DGR e produtividade, comparados ao controle (Tabela 3). Cavalcante et al. (2012) demonstraram que ao utilizar a relação entre dureza e alcalinidade >1 , há melhora no crescimento de juvenis de tilápia do Nilo. Além disso, o bom desempenho de crescimento para os tratamentos com elevação da dureza também pode ser atribuído a qualidade nutricional dos bioflocos, devido ao aumento do teor de aminoácidos essenciais em bioflocos menores (Ekasari et al. 2014). Entretanto, a FCR não demonstrou diferença entre os tratamentos. Esse parâmetro quantifica a sustentabilidade do sistema de produção, e nossos resultados demonstraram uma grande eficiência em relação ao uso dos nutrientes em sistema BFT, com FCA em torno de 1.0.

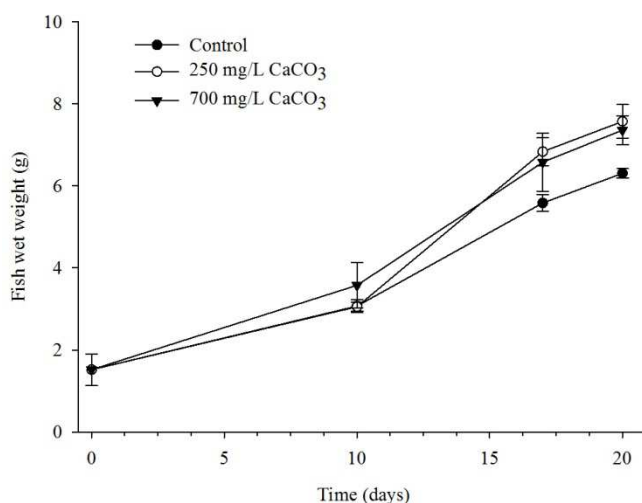


Fig. 4. Curva de crescimento de tilápia do Nilo *Oreochromis niloticus* durante berçário em sistema de bioflocos, utilizando níveis de dureza (controle, 250 750 mg L^{-1} CaCO_3).

Tabela 3 Desempenho de crescimento e utilização alimentar de tilápia do Nilo *Oreochromis niloticus* durante berçário em sistema de bioflocos utilizando níveis de dureza (controle, 250 750 mg L⁻¹ CaCO₃). Peso e comprimento inicial (1,51 ± 0,38 g e 4,05 ± 0,23 cm), estocados em caixas de 37,5 L a 30 peixes por caixa, durante 20 dias.

	Controle	250	700
Peso final (g)	6,30 ± 0,11 a	7,57 ± 0,40 b	7,37 ± 0,35 b
SGR (g/dia)	0,23 ± 0,05 a	0,30 ± 0,02 b	0,29 ± 0,01 b
Taxa de sobrevivência (%)	73,33 ± 10,00	85,55 ± 3,84	84,44 ± 5,09
Produtividade (kg/m³)	3,64 ± 0,43 a	4,85 ± 0,22 b	4,65 ± 0,05 b
FCR	1,15 ± 0,21	0,89 ± 0,03	0,93 ± 0,01

Dados representam média ± D.P. (Tukey, $p < 0.05$). SGR – taxa de crescimento específico; FCR – taxa de conversão alimentar.

Conclusão

A utilização de água com dureza elevada demonstrou melhor desempenho de crescimento e produtividade de tilápias do Nilo. Embora no início do ensaio a segunda etapa da nitrificação tenha sido um pouco mais lenta, após o completo estabelecimento não houve influência da dureza da água. Entretanto, pode ser recomendado a manutenção de até 250 mg L⁻¹ de CaCO₃ de dureza total, pois ao utilizar 700 houve aumento excessivo na produção de TSS e acentuada redução do FVI (alta densidade), sendo necessário aumentar a frequência das clarificações.

Futuros estudos relacionados ao reuso da água e a alteração iônica do meio são necessários para demonstrar a relação com a composição microbiana e o desempenho produtivo dos peixes.

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DISCUSSÃO GERAL

O intenso consumo de alcalinidade, e conseqüente redução do pH, tem sido relatado por estudos prévios com tilápia do Nilo *Oreochromis niloticus* em sistema BFT (Azim & Little 2008, Pérez-Fuentes et al. 2016). Entretanto, para a utilização desse sistema, é fundamental o correto manejo da alcalinidade e do pH, principalmente quando em alta densidade de estocagem.

O presente estudo demonstrou melhor desempenho de crescimento quando utilizados NaHCO_3 , comparado ao CaCO_3 , enquanto o Ca(OH)_2 foi similar aos demais tratamentos. Estudos prévios com camarão marinho *Litopenaeus vannamei* em BFT demonstram bom desempenho quando utilizados o NaHCO_3 ou Ca(OH)_2 (Furtado et al. 2011, Zhang et al. 2015), sendo este uma boa alternativa devido ao baixo custo (Furtado et al. 2014).

A tilápia do Nilo é considerada uma espécie que tolera um amplo intervalo de pH, entre 4,0–11,0 (Mahdi 1973). Entretanto, resultados heterogêneos tem sido demonstrados para o crescimento de tilápias do Nilo, como o intervalo de pH 4,0–6,0 em viveiros escavados (Rebouças et al. 2015) ou pH 7,0–8,0 de juvenis em aquários (El Sheriff & El-Feky 2009). Nossos resultados demonstraram desempenho superior de crescimento quando as tilápias foram mantidas em pH 7,5, comparado ao 8,3, enquanto o tratamento 6,5 foi similar aos demais.

O desempenho inferior de crescimento ao utilizar CaCO_3 ou pH 8,3 foi atribuído ao excessivo aumento dos sólidos suspensos totais, e redução do teor protéico dos bioflocos. Há uma estreita relação entre o manejo dos sólidos suspensos e o conteúdo nutricional dos bioflocos. Ray et al. (2010) estudando camarões marinhos *Litopenaeus vannamei*, demonstraram que ao aumentar a freqüência de clarificações, ocorre remoção de nutrientes do sistema, podendo induzir a redução no conteúdo protéico dos bioflocos.

O controle dos sólidos suspensos é um desafio em sistemas de alta densidade sem renovação de água, devido à grande entrada de ração no sistema. Como por ex., no presente estudo a produtividade final dos ensaios que testaram as fontes alcalinizantes e os níveis de pH, atingiu aprox. 25 kg/m³, e isso representa a entrada de aprox. 1,0 kg de ração/m³/dia. Portanto, é necessário demonstrar práticas de manutenção que possam reduzir ao máximo a produção de sólidos suspensos, pois além de alterar a composição

nutricional, as clarificações implicam em aumento dos custos de produção e geração de resíduos (*sludge*).

Ao avaliar a correção da dureza total, utilizando o controle (sem correção) e os níveis de 250 e 750 mg/L CaCO₃, houve crescimento superior para os tratamentos. O melhor crescimento pode ser atribuído a dois fatores: (1) aumento no teor de aminoácidos essenciais e (2) redução de gasto energético com a osmorregulação devido à perda iônicas.

O aumento do nível de Ca⁺² dissolvido resulta em redução do tamanho dos bioflocos (Luo et al. 2013). Enquanto Ekasari et al. (2014) demonstraram que bioflocos de tamanho menor possuem conteúdo protéico inferior, entretanto, o teor de aminoácidos essenciais é superior, comparado aos maiores.

Além disso, o Ca⁺² é relacionado ao aumento da coagulação-floculação em sistema de tratamento que utilizam lodo ativado (Peeters et al. 2011, Ye et al. 2016). Assim, a maior concentração de Ca⁺² pode ser utilizada para estabilizar o índice de volume de floco (FVI), que para aquicultura pode ser recomendado entre 40-100. Esse intervalo demonstra bioflocos com boa capacidade de sedimentação, como também capacidade de permanecer na coluna da água devido à intensidade de mistura (aeração) do sistema.

No aspecto fisiológico dos peixes, o Ca⁺² possui importante função diminuindo a abertura das *tight junctions* nas brânquias, o que evita as perdas iônicas (Wood 2001) e pode reduzir o gasto energético com a osmorregulação (Boeuf & Payan 2001). Ainda mais, quando em pH acidificado há aumento na permeabilidade branquial, causando maior perda iônica para o meio, e a elevação da dureza é uma estratégia utilizada para que isso seja evitado (Baldisserotto 2011).

Os alcalinizantes testados foram eficientes na correção da alcalinidade e do pH, e não houve diferença na ciclagem do nitrogênio inorgânico dissolvido (TAN e NO₂⁻). Enquanto ao utilizar pH 6,5 ou dureza total de 250 ou 700, ocorreu uma redução temporária na velocidade da segunda etapa da nitrificação (NO₂⁻ → NO₃⁻). Isso pode ser atribuído ao meio desfavorável para o crescimento das bactérias, necessitando mais tempo para a adaptação e o estabelecimento desses microorganismos. Esses são os primeiros resultados para a nitrificação em sistema BFT em água doce avaliando níveis de pH e dureza total, e são abordagens que devem ser mais exploradas.

Como forma de reduzir o consumo de composto alcalinizante, pode ser recomendado o uso de Ca(OH)_2 , em pH 6,5 e dureza total de até 250 mg/L. Embora não tenha sido avaliado, os resultados permitem inferir que essa configuração pode representar consumo de alcalinizante em relação à ração entre 5-8%. Essa relação pode ser utilizada para estimar o acúmulo iônico no sistema, como também para calcular o custo com o alcalinizante no ciclo de produção.

Os resultados demonstram aumento de Na^+ ou Ca^{+2} ao longo do ensaio, obviamente de acordo com o alcalinizante utilizado. Entretanto, no presente estudo esses acúmulos não parecem ter comprometido o desempenho de crescimento dos peixes. Esses resultados indicam a necessidade de monitoramento das concentrações iônicas, especialmente quando o sistema opera em alta densidade de estocagem, por longos períodos de tempo e/ou reutilizando a água.

A avaliação do status fisiológico e bioquímico dos peixes em sistemas superintensivos é fundamental, pois são ferramentas utilizadas para detectar os possíveis distúrbios causados aos organismos cultivados. No presente estudo, quando utilizadas as diferentes fontes alcalinizantes ou níveis de pH, não foram detectadas alterações dos parâmetros hematológicos (glicose, hematócrito e osmolalidade). Ainda mais, os valores permaneceram dentro do intervalo considerado normal para tilápia do Nilo (Bittencourt et al. 2010).

A capacidade antioxidante é determinada pela capacidade de bloquear os efeitos das espécies reativas de oxigênio, e quando não é efetiva, ocorre o estresse oxidativo (Jones 2006). O estresse oxidativo tem sido utilizado para avaliar as condições de cultivo em aquicultura, relacionado aos níveis de amônia (Sinha et al. 2014) e hipóxia (Welker et al. 2013). Ao avaliar os parâmetros oxidativos (ACAP, LPO, SOD e CAT), não ocorreram alterações ao utilizar as diferentes fontes alcalinizantes (NaHCO_3 , CaCO_3 ou Ca(OH)_2) ou os níveis de pH (6,5, 7,5 ou 8,3). Isso indica que as condições de cultivo não impuseram desafio fisiológico aos peixes, pois estão dentro do intervalo de tolerância da espécie.

Finalmente, durante todos os ensaios não houve renovação de água, apenas reposições por perdas com evaporação e clarificação. Além disso, as taxas de conversão alimentar permaneceram em torno de 1,0 e a sobrevivência dos peixes em aprox. 80%. Esses resultados demonstram a eficiente utilização de água e nutrientes, atingindo

excelente desempenho produtivo, o que confirma a sustentabilidade da tecnologia dos bioflocos.

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CONCLUSÃO GERAL

A presente tese demonstra temas ainda pouco explorados para a produção de tilápias do Nilo em BFT. As conclusões são listadas abaixo.

- A utilização de NaHCO_3 ou Ca(OH)_2 proporciona o melhor desempenho de crescimento e produtividade em sistema BFT para juvenis de tilápias do Nilo, sem comprometer a nitrificação ou o status fisiológico dos peixes. Economicamente, o uso de Ca(OH)_2 pode ser mais vantajoso que NaHCO_3 , devido ao menor valor de mercado e utilização relativa à quantidade de ração (7 vs 14%, respectivamente).
- Iniciar a formação dos bioflocos com pH próximo a 8,0 representa vantagens à nitrificação. Entretanto, após o estabelecimento da nitrificação, pode ser recomendado a manutenção do sistema em pH 6,5. Isso representa menor utilização de alcalinizante em relação à ração utilizada, comparando o pH 6,5 ao 8,3 (13 vs 20%, respectivamente). Consequentemente, isso contribui para a preservação da composição iônica da água, reduzindo a produção de sólidos suspensos totais e a quantidade de remoções de flocos. Além disso, utilizar pH 6,5 proporciona aumento no teor protéico dos bioflocos.
- A elevação da dureza total entre 250 e 700 mg/L CaCO_3 demonstrou desempenho de crescimento e produtividade maiores que o controle. Entretanto, há uma relação positiva entre a dureza e o aumento dos sólidos suspensos, devido a adesão do Ca^{+2} às substâncias poliméricas extracelulares (EPS) produzidas pelas bactérias. Consequentemente ocorre redução do tamanho e aumento da densidade (menor FVI) dos bioflocos. Esses resultados podem ser benéficos devido a três aspectos: (1) melhora da composição nutricional, (2) evita a redução excessiva da densidade (*bulking*, $\text{FVI} \approx 200 \text{ mL/g}$) dos bioflocos e (3) demonstra ser possível o reuso da água mesmo quando a água é extremamente dura (até 700 mg/L CaCO_3).
- O monitoramento ao longo do cultivo dos níveis de NO_3^- , PO_4^- , e os íons Na^+ , Ca^{+2} e Cl^- são fundamentais, pois por um longo prazo, podem atingir níveis capazes de causar efeitos deletérios ao crescimento.

- Finalmente, ao analisar conjuntamente os resultados, é possível concluir que para atingir o máximo desempenho de crescimento para juvenis de tilápia do Nilo em sistema BFT, utilizando peixes com peso inicial entre 1,5 - 3,0 g, densidade inicial de 670–800 peixes m³ ou 1,3–1,6 kg/m³ e densidade final até 25,0 kg/m³, é necessário manter as seguintes condições:
 - Utilizar como composto alcalinizante: NaHCO₃ ou Ca(OH)₂;
 - intervalo de pH: 6,5–7,5;
 - intervalo de dureza total: 250–700 mg/L CaCO₃;
 - sólidos suspensos totais: <800 mg/L
 - índice de volume do floco (FVI): 40–80 mL/g.