UNIVERSIDADE FEDERAL DE MINAS GERAIS

**SALINIDADE DA ÁGUA DURANTE A MASCULINIZAÇÃO**
**DE TILÁPIA DO NILO (*Oreochromis niloticus*)**
**EM SISTEMA DE BIOFLOCOS**

RAFAEL CAVACA ALVES DO VALLE

BELO HORIZONTE
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Dissertação apresentada ao Programa de Pós-graduação em Zootecnia da Escola de Veterinária da Universidade Federal de Minas Gerais como requisito parcial para obtenção do título de Mestre em Zootecnia

Área de concentração: Produção Animal

Prof. Orientador: Dr. Eduardo Maldonado Turra

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## FOLHA DE APROVAÇÃO

**"Salinidade da água durante a masculinização de tilápia do nilo (*Oreochromis niloticus*) em sistema de bioflocos"**

**Rafael Cavaca Alves do Valle**

Dissertação de Mestrado defendida e aprovada, no dia vinte e nove de abril de dois mil e vinte pela Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Zootecnia da Universidade Federal de Minas Gerais, constituída pelos seguintes professores:

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DISSERTAÇÃO defendida e aprovada em 29/04/2020 pela Comissão Examinadora composta pelos seguintes membros:

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Prof. (Orientador) Dr. Eduardo Maldonado Turra

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Prof. Dr. Galileu Crovatto Veras

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Prof. Dr. Thiago Bernardes Fernandes Jorge

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# RESUMO GERAL

O objetivo do presente trabalho foi avaliar a salinização da água para a masculinização de tilápias do Nilo (*Oreochromis niloticus*) em sistema de bioflocos. Sete níveis de salinidade (0, 2, 4, 6, 8, 10 and 12 g/L) foram testados em larvas de tilapia pós-absorção do saco vitelino, por quatro semanas (período de masculinização) em flocos maduros. Não houve diferenças entre os tratamentos para temperatura, oxigênio dissolvido, sólidos sedimentáveis, sólidos totais e nitrato. Nitrogênio ammoniacal total foi maior que 1 mg/L para quase todos os tratamentos, entretanto, as médias para amônia tóxica permaneceram abaixo do limiar tóxico. O pH, a alcalinidade e o nitrito aumentaram juntamente com os níveis de salinidade. Os picos de nitrito durante o experiment foram maiores em níveis de salinidade mais altos também, negativamente correlacionados com os resultados de sobrevivência. Biomassa final e conversão alimentar apresentaram redução e aumento, respectivamente, com o incremento da salinidade. A taxa de sobrevivência diminuiu com o aumento da salinidade e a taxa de masculinização tendeu a reduzir também. Portanto, a salinidade deve ser mantida próximo de 0 g/L no protocolo de masculinização de larvas de tilápia do Nilo em ambiente de BFT, para melhor sobrevivência e conversão alimentar e garantir maior taxa de masculinização.

Palavras-chave: Cloreto de sódio, inversão sexual, larvicultura, tecnologia de bioflocos.

# ABSTRACT

The aim of the present study was to evaluate water salinity for Nile tilapia (*Oreochromis niloticus*) in bioflocs during masculinization. Seven salinity levels (0, 2, 4, 6, 8, 10 and 12 g/L) were tested in tilapia after absorption of the yolk sac, for four weeks (masculinization period), in a matured bioflocs. There was no difference between treatments for temperature, dissolved oxygen, settleable solids, total suspended solids and nitrate. Total ammonia was bigger than 1 mg/L for almost all treatments, however, the averages of toxic ammonia remained below the toxicity threshold. The pH, alkalinity and nitrite variables increased with higher salinity levels. The nitrite peaks were bigger for the higher salinities too, in addition to a significant negative correlation with the survival rate. Final biomass and feed conversion demonstrated a reduction and an increase, respectively, with the increase in salinity. The survival rate decreased with the increase of salinity and the masculinization rate tends to reduce too. Therefore, salinity should be kept close to 0 g/L in the masculinization protocol of Nile tilapia larvae in BFT, for a better survival, food conversion ratio and to guarantee higher masculinization rate.

Keywords: Sodium chloride, sexual inversion, larviculture, biofloc technology.

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## 1. INTRODUÇÃO

A aquicultura trata da criação ou cultivo controlado de organismos aquáticos tais como peixes, moluscos, crustáceos e plantas, de água doce ou salgada. Além de ser uma atividade muito antiga, ela é difundida em todo o mundo, dividindo-se em dois segmentos: o cultivo de organismos direcionados para a alimentação humana e animal e o cultivo para fins ornamentais. Desde os primórdios da atividade até os dias de hoje, muitas tecnologias de cultivo surgiram, como, por exemplo, os sistemas extensivo e intensivo em viveiros escavados, tanques-rede em reservatórios, recirculação em galpões cobertos, aquaponia em caixas d'água e bioflocos salinizados em estufas agrícolas.

Esse último exemplo, o sistema de tecnologia em bioflocos (BFT) foi desenvolvido inicialmente na década de 1970 para a produção de camarão marinho com troca mínima de água, e nas décadas de 80 a 90 também desenvolvido para peixes, de acordo com Emerenciano et al. (2013). Atualmente, segundo Durigon (2018), o uso dessa tecnologia tem sido investigado para várias espécies de peixes, como *Brycon orbignyanus* (Sgnaulin et al., 2018), *Piaractus brachypomus* (Chaverra-Garcés et al., 2017), *Rhamdia quelen* (Poli et al., 2015), *Mugil cf. hospes* (Rocha et al., 2012), *Cyprinus carpio* (Zhao et al., 2014) e tilápia do Nilo *Oreochromis niloticus* (Luo et al., 2014; Long et al., 2015). Segundo Fitzsimmons et al. (2011), a tilápia do Nilo é conhecida como uma espécie de rápido crescimento, rusticidade, fácil adaptação a sistemas intensivos e boa aceitação de dietas comerciais.

O aumento da população mundial, bem como a preocupação, cada vez mais difundida, com a fonte de proteína para a alimentação humana, além da necessidade de preservação dos recursos naturais, em condições que permitam as gerações futuras de perpetuarem-se no planeta Terra, levaram ao uso de sistemas de aquicultura intensivos que possibilitam um melhor aproveitamento de recursos como água, terra, luz natural e energia elétrica.

Atualmente, a tilapicultura em tanques suspensos, dentro de estufas e com tecnologia de bioflocos salinizados está longe de ser o sistema mais difundido no Brasil, mas demonstra um excelente potencial. A tecnologia de bioflocos (BFT) apresentou resultados positivos em carcinicultura com *Litopenaeus vannamei* (Esparza-Leal et al., 2016; Khatoon et al., 2016; Xu et al., 2015; Abreu et al., 2019), *Penaeus monodon* (Huang et al., 2016) e *Macrobrachium rosenbergii* (Miao et al., 2017). Também em

piscicultura com *Oreochromis niloticus* (Day et al., 2016; Lima et al., 2018) e *Clarias gariepinus* (Ekasari et al., 2016).

Os bioflocos são compostos principalmente de bactérias, microalgas, fezes, matéria orgânica em decomposição, protozoários, cianobactérias, pequenas larvas de metazoários e invertebrados e outros microrganismos (Avnimelech, 2009). Os bioflocos são ricos em nutrientes, como vitaminas e minerais, têm efeitos probióticos (Hargreaves, 2013) e, portanto, são benéficos para as espécies cultivadas (Silva et al., 2013). Eles também são uma boa fonte de proteína, variando de 25% a 50% do seu peso, e de lipídios, que variam de 1% a 5% do seu peso (Hargreaves, 2013; Wang et al., 2015). Pérez-Fuentes et al. (2016) demonstraram ser possível alcançar uma produtividade de 18 kg.m$^{-3}$ de tilápia nilótica, com sobrevivência de 95% em um sistema de bioflocos, utilizando uma relação C:N de 10:1. Segundo Zhang et al. (2016), essa produtividade, na tilapicultura, pode ser maior (37,93 kg.m$^{-3}$) quando a fonte de carbono é o ácido poli-β-hidroxibutírico. Dessa forma, a tilápia do Nilo criada em sistema de bioflocos apresentou resultados satisfatórios em termos de crescimento e sobrevivência.

Além dos excelentes resultados de produção, a tilápia ainda apresenta outras vantagens ou pontos positivos. Ela é bem aceita pelo mercado consumidor global, devido ao seu filé sem espinhas intramusculares, carne branca e textura firme (Simões et al., 2007). Além disso, de acordo com Rodrigues et al. (2015), a tilápia é um peixe filtrador que pode consumir organismos presentes nos bioflocos. Isso contribui muito para a sua adaptação e desempenho em BFT, pois esse sistema possui alimentos vivos ricos em nutrientes disponíveis em tempo integral para os peixes (Martínez-Córdova et al., 2015). Segundo Souza et al. (2019), outro fator que deve ser levado em conta é a sua adaptação a águas com moderada salinidade, o que é de suma importância na tecnologia de bioflocos e que reflete num desempenho zootécnico superior dos espécimes cultivados sob essa condição. Isso permite, inclusive, o policultivo com camarão, ou mesmo, monocultivo de tilápias em fazendas de camarão que não estão operando devido à propagação de doenças que acometem a carcinicultura.

Dentro do conceito de bioflocos salinizados, entende-se que o nível de salinidade pode ser descrito como a incorporação de Cloreto de Sódio (NaCl) na água de cultivo de peixes, o que pode melhorar o metabolismo e os processos osmoregulatórios em peixes, além de aumentar a sobrevivência durante eventuais picos de nitrito, quando comparado com sistemas não salinizados, em função da competição, pelos mesmos sítios de ligação, entre o íon cloreto e o nitrito. Entretanto, não há muita informação na literatura

sobre qual a melhor salinidade da água de cultivo para a fase de larvicultura da tilápia do Nilo e para o seu procedimento de masculinização em sistema de bioflocos. Para o sistema de bioflocos, na verdade, desconhecemos literatura publicada para esta fase específica. Dessa forma, a proposta do presente trabalho consiste em determinar qual a melhor concentração de sal na água durante a masculinização em sistema de bioflocos.

## 2. REVISÃO DE LITERATURA

### 2.1. Bioflocos

O sistema de bioflocos baseia-se na retenção de resíduos de fezes e sobras de ração, com agitação e aeração constante para manter os sólidos em suspensão e o nível de oxigenação adequado (Azim e Little, 2008; Liu et al., 2014), e com a mínima troca de água do sistema (Crab et al., 2010). Neste sistema, microrganismos preferencialmente aeróbicos e heterotróficos utilizam o carbono orgânico (residual e suplementado) como fonte de energia para transformar o nitrogênio inorgânico na forma de amônia, que é tóxica para os peixes, para produzir suas proteínas celulares (Crab et al., 2010; De Schryver et al., 2008). Essas proteínas podem ser utilizadas na alimentação de espécies de peixes filtradores, como a tilápia (Crab et al., 2012).

Os flocos microbianos são formados por um agregado de organismos: bactérias, partículas orgânicas, protozoários e algas (De Schryver et al., 2008). Esses flocos apresentam formas variadas, com tamanho médio entre 50 a 200 μm (Azim e Little, 2008). O tamanho do floco é um meio de avaliação da qualidade do floco, segundo Ekasari et al. (2015), flocos maiores que 100 μm apresentaram maiores concentrações de proteína e lipídeos, enquanto flocos menores que 48 μm apresentaram maiores quantidades de aminoácidos essenciais e melhores taxas de assimilação de N, possivelmente devido a sua melhor digestibilidade e facilidade de ingestão.

Segundo Luo et al. (2014) e Azim e Little (2008), os flocos microbianos promovem redução da conversão alimentar e aumento no peso final em animais filtradores. O aproveitamento do floco pode corresponder a 50% da alimentação diária (cerca de 2% do peso vivo) e ainda a taxa de assimilação diária de 0,3 g de N por kg, equivalente a 2 g de proteína por dia, na fase inicial de cultivo (Avnimelech, 2007), mostrando que a filtração é uma fonte adicional de nutrientes na dieta.

O nome "Bioflocos" advém da coagulação de proteínas e micro-organismos por meio das constantes mitoses bacterianas que utilizam o C e o N como matérias-primas. O floco por sua vez, serve de alimento para determinados crustáceos e peixes filtradores (Alvimelech, 2009; Schneider et al., 2005; Crab et al., 2012; Turra et al., 2016). É possível trabalhar com o BFT ao fornecer constante aeração e agitação da água, permitindo assim um importante processo desse sistema: a decomposição aeróbica pelas bactérias heterotróficas (Azim e Little, 2008). A comunidade heterotrófica é predominante no sistema devido a sua menor DBO (demanda biológica de oxigênio), superior taxa de crescimento e maior produção de biomassa por unidade de substrato em

comparação com as bactérias nitrificantes (Hargreaves, 2006; Azim e Little., 2008; Crab et al., 2012). Apesar disso, as bactérias nitrificantes são responsáveis por converterem a amônia em nitrito e o próprio nitrito em nitrato, reduzindo, assim, consideravelmente, o potencial toxicológico de nitrogenados oriundos da degradação proteica.

### 2.2. Criação de tilápias em BFT

A tecnologia dos sistemas de bioflocos claramente contribui para o crescimento e a produção de peixes filtradores, como a tilápia, seja em sistemas fechados com luminosidade limitada (Azim e Little, 2008) ou estufas com exposição à luz solar (Day et al., 2016). A maioria dos estudos apontam um significante aumento do desempenho zootécnico de animais cultivados em Sistemas de Bioflocos (Azim e Little, 2008; Xu e Pan, 2012; Luo et al., 2014).

Esse fato pôde ser comprovado também em estudo de reprodução e larvicultura, onde Ekasari et al. (2015) concluíram que larvas de tilápia do Nilo, oriundas de matrizes em BFT, cultivadas em BFT por 14 dias, apresentaram taxa de sobrevivência superior em relação a reprodutores cultivados em água clara.

Day et al., (2016) divulgaram um estudo com três espécies de tilápias (*O. mossambicus, O. andersonii e O. niloticus*) em BFT. A tilápia do Nilo se destacou, apresentando inferior conversão alimentar (1,0) quando comparada às demais (>2,0). A espécie nilótica também demonstrou ganho de peso superior, de 0,693 g/dia, contra 0,405 e 0,185 g/dia de *O. mossambicus e O. andersonii*, respectivamente. É interessante ressaltar que nesse estudo foi possível confirmar a informação divulgada por Skelton (2001) de que das três espécies, cada uma se alimenta em níveis tróficos diferentes, confirmando a hipótese de que uma delas demonstraria superior desempenho em bioflocos.

### 2.3. Formação de lotes monossexo

A engorda de tilápias para abate, seja em BFT ou não, deve ser feita com lotes masculinos, porque eles possuem uma produtividade superior quando comparados aos lotes mistos (Beardmore et al., 2001; Palaiokostas et al., 2015). É notório que machos desse grupo de espécies cresçam significativamente mais rápido que as fêmeas (El Sayed, 2006; Sun et al., 2016). Em lotes monosexo não há reprodução e ocorre eliminação de comportamentos que limitam o crescimento dos peixes (Beardmore et al., 2001). Desta forma, observa-se no cultivo de lotes monosexo masculinos: altos índices

de crescimento, menor conversão alimentar, alta tolerância a condições adversas no ambiente, uniformidade do lote, melhor aparência e qualidade do filé, resistência a doenças e ao estresse e redução da agressividade (El-Sayed, 2006; Mlalila et al., 2015). Salienta-se que caso haja fuga dos animais em sistema de tanques-rede, a possibilidade de uma eventual reprodução e propagação da espécie também é menor (Beardmore *et at.*, 2001; Singh, 2013).

Para se obter os lotes monossexos, com maior precisão e eficiência, é necessário conhecer os processos fisiológicos, embriológicos e genéticos envolvidos na diferenciação e determinação sexual dos peixes. A tilápia do Nilo (*Oreochromis niloticus)* é um peixe gonocorístico, no qual os indivíduos crescem e diferenciam como machos ou fêmeas e permanecem dessa forma durante toda sua vida (Devlin, 2002). Os primeiros sinais da diferenciação sexual em pós-larvas de tilápia ocorrem entre 23 a 26 dias com a formação da cavidade ovariana em indivíduos XX, no caso fêmeas, ou a formação de ductos eferentes, nos machos XY. Entretanto, o período crítico para a diferenciação da gônada é dos 5 a 10 dias pós eclosão (dpe).

### 2.4. Salinidade na larvicultura

A salinidade, em um corpo d'água, se deve à presença de sais dissolvidos que representam 60 dos 92 elementos químicos básicos da natureza. A salinidade é um fator determinante no controle do crescimento dos peixes (Riley, 1965; Boeuf e Payan, 2001). A osmorregulação pode levar a um maior gasto energético na regulação dos fluidos corporais dos peixes (10 a 50% do balanço energético) (Boeuf e Payan, 2001), o que pode ter consequências negativas em relação ao consumo de oxigênio, alimentação e regulação hormonal, interferindo diretamente no desempenho das espécies cultivadas (Souza *et al.,* 2019). De acordo com Grau *et al.* (1994), os peixes possuem células prolactínicas, as quais são, diretamente, osmosensitivas. Eles também possuem, segundo Laurent e Dunel-Erb (1984), quimiorreceptores pseudobranquiais que fornecem informações sobre a salinidade da água. A osmorregulação é fundamental para a sobrevivência e desempenho dos peixes, não só devido à regulação de fluidos corporais, mas também à produção de muco na pele, que age, fisiologicamente, como uma barreira imunologicamente ativa na proteção contra microrganismos oportunistas e patogênicos presentes no ambiente de cultivo.

De acordo com Altinok e Grizzle (2001), a salinidade da água pode influenciar positivamente o metabolismo e os processos osmorregulatórios em peixes e favorecer

seu desempenho em gradientes adequados. O sal é um produto permitido por lei utilizado na aquicultura continental e quando utilizado na concentração correta para espécie pode resultar em maior ou semelhante sobrevivência que em ambientes de água doce (McDonald et al., 2015).

Luz et al. (2013) ao avaliar o efeito de diferentes salinidades da água na larvicultura de tilápia (*O. niloticus*) sobre a sobrevivência e desempenho das larvas durante 30 dias, concluíram que larvas mantidas a 6 g/L apresentaram mortalidade total, antes de dez dias de alimentação ativa. Os autores concluíram que a larvicultura de tilápia pode ser realizada em água salinizada a 2 g/L, com resultados de desempenho e sobrevivência semelhantes aos registrados em água doce.

Diversos estudos mostraram que esta espécie pode ser mantida em concentrações de sal superiores durante a fase juvenil e adulta (Kamal e Mair, 2005; El-Sayed et al., 2005; Chowdhury et al., 2006). Informação também sugerida por Alvarenga et al. (2018), que avaliaram o crescimento, sobrevivência, lesões branquiais e composição de filé de alevinos de *Oreochromis niloticus* criados durante 70 dias com diferentes salinidades (0, 4, 8, 12 e 16 g/L) em bioflocos maduros. Ainda de acordo com Alvarenga et al. (2018), a água salinizada, especialmente entre 4 e 8 g/L (ótimo em torno de 6 g/L), pode ser recomendada em BFT para melhorar o desempenho da tilápia na fase inicial da cultura. O nível de salinidade avaliado não afetou a composição do filé, nem a ocorrência de lesões branquiais.

No entanto, os resultados do trabalho de Luz et al. (2013) mostraram que larvas de tilápia, com cinco dias pós-eclosão, não toleram a transferência direta da água doce para a salinidade de 6 g/L. Parte da solução deste entrave pode ter sido apresentado por Larumbe-Morán et al. (2010), que ao utilizarem tilápias com peso médio inicial de 0,25 g, verificaram que estes animais puderam ser adaptados até a salinidade de 25 g/L, por um processo gradual de aumento diário de salinidade de 5 g/L. Um processo gradual de adaptação, com resultados satisfatórios também foi obtido por Lemarie et al. (2004), que ao trabalharem, na fase de larvicultura, com taxas de adaptação de 2, 4, 6, 8, 10, 12 e 14 gramas por litro por dia, concluíram que o melhor resultado na análise de regressão foi de 2,5 g/L/d.

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## 3.    ARTIGO

**Title:** Water salinity during masculinization of Nile tilapia (*Oreochromis niloticus*) in biofloc system

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**Abstract**

The aim of the present study was to evaluate water salinity for Nile tilapia (*Oreochromis niloticus*) in bioflocs during masculinization. Seven salinity levels (0, 2, 4, 6, 8, 10 and 12 g/L) were tested in tilapia after absorption of the yolk sac, for four weeks (masculinization period), in a matured bioflocs. There was no difference between treatments for temperature, dissolved oxygen, settleable solids, total suspended solids and nitrate. Total ammonia was bigger than 1 mg/L for almost all treatments, however, the averages of toxic ammonia remained below the toxicity threshold. The pH, alkalinity and nitrite variables increased with higher salinity levels. The nitrite peaks were bigger for the higher salinities too, in addition to a significant negative correlation with the survival rate. Final biomass and feed conversion demonstrated a reduction and an increase, respectively, with the increase in salinity. The survival rate decreased with the increase of salinity and the masculinization rate tends to reduce too. Therefore, salinity should be kept close to 0 g/L in the masculinization protocol of Nile tilapia larvae in BFT, for a better survival, food conversion ratio and to guarantee higher masculinization rate.

Keywords: Sodium chloride, sexual inversion, larviculture, biofloc technology.

## 3.1. INTRODUCTION

The technology of bioflocs (BFT) was initially developed in the 1970's for the production of marine shrimp with minimal water exchange, and in the 1980's and 1990's also for fish (Emerenciano et al., 2013). Currently, according to Durigon (2018), the use of this technology has been investigated for several species of fish, among them *Oreochromis niloticus* (Luo et al., 2014; Long et al., 2015). Nile tilapia is recognized as a species of rapid growth, rusticity, easy adaptation to intensive systems and good acceptance to commercial diets (Fitzsimmons et al., 2011).

BFT are mainly composed of bacteria, microalgae, feces, decomposing organic matter, protozoa, cyanobacteria, small metazoan larvae, invertebrates, and other microorganisms (Avnimelech, 2009). They are rich in nutrients, such as vitamins and minerals, have probiotic effects (Hargreaves, 2013) and, therefore, are beneficial for

cultivated species (Silva et al., 2013). They are also a good source of protein, ranging from 25% to 50% of their dry weight, and lipids, which range from 1% to 5% (Hargreaves, 2013; Wang et al., 2015).

Within the concept of salinized BFT, it is understood that the level of salinity can be described as the incorporation of sodium chloride (NaCl) in the fish farming water, which can improve metabolism and osmoregulatory processes. In addition, water salinity may increase survival during eventual nitrite peaks, when compared with non-salinized systems, due to the competition, for the same binding sites, between the chloride ion and nitrite. Alvarenga et al (2017) indicated the salinized water, especially between 4 and 8 g/L, is recommended in BFT to improve the growth performance of Nile tilapia in the initial culture phase (tilapia from 0.5 to 20 g). However, there was not much information in the literature about the best salinity for the larval farming and masculinization of Nile tilapia in a BFT. The aim of the present study was to evaluate different water salinities concentrations during masculinization of Nile tilapia in BFT.

### 3.2. MATERIAL AND METHODS

The experiment was carried out in an agricultural greenhouse at Aquaculture Laboratory (LAQUA) of the Veterinary School of the Federal University of Minas Gerais (Universidade Federal de Minas Gerais - UFMG), Brazil. All procedures with animal manipulation were approved by the Ethics Committee on the Use of Animals (CEUA- UFMG), Protocol 312/2019.

### 3.2.1. Animals, facilities and experimental design

A completely randomized design with seven treatments and three replicates was used, totaling 21 experimental units. Seven distinct levels of sodium chloride salinity (0, 2, 4, 6, 8, 10 and 12 g $L^{-1}$) were established in the culture water of tilapia larvae (*Oreochromis niloticus*) until the end of the masculinization period, for four weeks, in a previously matured biofloc environment.

Each experimental unit was a 150 L tank equipped with a thermostat, set at 28°C, and air stones and hoses coupled to an air blower for constant aeration and water revolving. A total of 6,300 larvae were used, in which 300 Nile tilapia larvae were allocated per tank, after yolk sac absorption, with initial weight of approximately 0.01 g, at a density of 2 larvae/L. The post yolk sac absorption larvae were randomly collected

from at least 10 spawnings of females, that were from a large genetic basis of Nile tilapia broodstock (Chitralada line) of the Nucleus of Studies in Nutrition, Genetics, and Technology in Aquaculture (NGTAqua) of the Federal University of Minas Gerais (UFMG). This genetic line presents an inbreeding rate of less than 1.3% and was used to avoid a family × treatment interaction effect.

### 3.2.2. Feeding and masculinization

The larvae were fed for 28 days with a commercial ration (Propescado-Nutriave Foods)  containing 55% crude protein (CP) and enriched with the masculinizing hormone 17-alpha-methyltestosterone (17 MT) at a concentration of 60 mg/kg of feed (Silva, 2017; Baroiller and D'Cotta, 2018). There was correction of the ration supplied per week, starting with a daily treat of 30% of the biomass, decreasing to 25%, 20%, and 15% in the second, third, and fourth weeks, respectively. The animals received feed at a feeding frequency of eight times a day, hourly, from 08:00 am to 04:00 pm. The amount of feed was divided equally between feeding intervals. For logistical reasons, the animals were kept stocked, at the respective tanks and sodium chloride concentrations, receiving the same feed, but without hormone, for three more days, i.e. until day 31, when the experiment could be finished.

At the end of the experiment, 60 randomly selected fingerlings from each tank. The animals were weighed, euthanized by anesthetic overdose (300 mg $L^{-1}$ of eugenol) and immersed in Bouin's liquid. After 24 hours, the fingerlings were immersed in 70% alcohol and their gonads were stained with aceto carmine for the sexing technique proposed by Guerrero and Shelton (1974). The masculinization rate was estimated, calculating the number of males, established by microscopic evaluation, divided by the total number of animals evaluated, and multiplying the result by 100.

### 3.2.3. Water quality

Water quality (dissolved oxygen, temperature, pH, and salinity) was daily monitored using a multiparameter AKSO probe. Spectrophotometer readings of ammonia concentrations were taken three times a week, nitrite concentrations, twice a week, and nitrate concentrations, twice, once at the beginning and once at the end of the experiment. The alkalinity was checked also twice, once at the middle and once at the end of the experiment. Since the alkalinity values were  satisfactory, limestone was not

added in the tanks. Salinity was kept with common salt (NaCl) in treatments 2, 4, 6, 8, 10, and 12 g/L, and in treatment 0 g/L no salt was added. To reach salinity levels in treatments above 2 g/ L, an adaptation protocol of larvae was performed at a rate of 2 g/L per day, as established by Lemarié et al. (2004) to avoid high mortality.

When the total ammoniacal nitrogen (N-AT) concentration reached values higher than 1 mg/L, the sugar cane (organic carbon source) was added, at a rate of 6 g carbon for each 1 g N-AT (Ebeling et al., 2006). The settleable solids was evaluated by the Imhoff cone method (Avnimelech, 2007) five times throughout the experiment. Since the settleable solids level was satisfactory, no drainage was required. To obtain measurements of total suspended solids, 50 mL of water were weekly filtered in a synthetic polypropylene felts that were dried, weighed, and wrapped in aluminum foil, and after filtration, were sent to the oven at a temperature of 100ºC for 48 hours to be weighed on an analytical balance.

For the calculation of non-ionized ammonia (toxic ammonia), the methodology described by Emerson et al. (1975) was used:

$$pKa = 0.09018 + 2729.92 \times T^{-1}$$
$$f = 1 \times (10^{pKa - pH + 1})^{-1}$$

In which, pKa is the potential of the acidity constant or equal to - log Ka;

T is the temperature in degrees Fahrenheit;

f is the correction factor for total ammonia to calculate un-ionized ammonia;

pH is the potential of hydrogen.

### 3.2.4. Growth performance

At the end of the experiment feed consumption, final biomass, average final weight, feed conversion, masculinization rate, and survival rate were evaluated. All responses were obtained per experimental unit. The average final weight was the average weight of the randomly selected animals used for sexing in each tank. Biomass was the average weight multiplied by the number of animals at the end of experiment. Feed conversion was the amount of feed supplied divided by the final biomass produced in each tank. Survival was the number of animals alive after the end of the experiment divided by 300, multiplying the result by 100.

### 3.2.5. Statistics

The data were submitted to the R (R Core Team, 2016) and Infostat (Di Rienzo, et al., 2015) software for the appropriate analyses. Linear regressions were fitted, and the residuals of the models were checked for normality (Shapiro-Wilk test) and homogeneity (post-tested by SNK, according to Di Rienzo, et al., 2015) of variances assumptions. Nitrite data was transformed in natural logarithm to properly normalize the values and then submitted to ANOVA and to fit a linear regression model.

### 3.3. RESULTS AND DISCUSSION

Temperature is an important variable in all stages of tilapia production. Sudden fluctuations may be determinant in the animals' performance. According to El-Sayed (2006), it is recommended for the cultivation of Nile tilapia, a temperature of 28ºC. The acceptable temperature range for a development compatible with production systems varies from 25 to 30 ºC, with satisfactory results, since tilapia, according to FAO (2012), are warm water fish and tolerate a wide temperature range. In the present experiment, the averages of the temperature data remained within this optimum range for growth (table 1), and significant differences between treatments were not observed. The data found are similar to those described by Wedemeyer (1996) as recommended for the growth of tilapia.

Regarding dissolved oxygen (DO), according to Wedemeyer (1996) and Avnimelech (2009), for biofloc systems, a concentration above 4 mg/L is recommended. According to El-Sayed (2006), DO is a factor that limits the metabolism, food and growth of fish, in addition to production and survival (Avnimelech, 2009). Also, according to Avnimelech (2009), failures in the aeration system of the tanks can cause, in biofloc systems, sedimentation of floc and reduction of oxygen levels, in addition to increasing the mortality rate of the exposed population. In the research carried out, the DO of all treatments remained above 6 mg/L, with no statistical difference between treatments (table 1), in addition to being in accordance with the minimum of 5 mg/L suggested by Boyd and Tucker (2012) not to limit fish performance.

According to Avnimelech (2009), settleable solids should be kept below 100 mL/L. In the present experiment, the average values of treatments were below this reference value and also did not differ, statistically, from each other (table 1). This variable is important because it guides the practice of water exchange, that is, the need

to drain the floc and replace it with freshwater in the system. In this experiment there was no need to perform drainage, just the need for replacement by evaporation. The variable total suspended solids also remained within the values recommended by Avnimelech (2009), that is, below 1000 mg/L (table 1). This variable is important because due to BFT maturation, it is possible to increase the sedimentation of particles, which in turn, can, when in excess, lead to decrease food consumption, to reduce branchial function and even take to the fish to death (Zhu et al., 2016), mainly in fish larviculture, a more sensitive growth phase. Ultimately, this variable directly interferes with water quality, making it possible to worsen growth performance and productivity. The maximum mean value reached in a single treatment was 270 mg/L, with no statistical difference between treatments, which corroborates that the response remained at ideal levels.

The pH variable should assume values recommended by Wedemeyer (1996), which range from 6 to 9 on pH scale. According to Rebouças et al. (2016), different pH levels, in a range of 5.5 to 9.0, did not result in changes in the final weight, specific growth rate and feed conversion of Nile tilapia. According to Avnimelech (2009), the pH must be kept in a range from 7 to 9. In the present experiment, it was verified an amplitude of the average values from 7.76 to 8.27 (table 1). These values are within the recommended range, although the pH values increased with higher salinity levels, there were no biological implication. In addition, there was a decrease in pH values over the weeks of the experiment (figure 1). This behavior is expected in bioflocs and could be evidenced by Azim and Little (2008), Alves et al. (2017) and Alvarenga et al. (2018). This reduction occurs due to the demand for carbonate and bicarbonate ions by the autonomous microbiota of the biofloc system, which leads to a consumption of calcium carbonate and a reduction in pH levels (Ebeling et al., 2006). However, $CaCO_3$ concentration was remained above 50 mg/L, throughout the experiment, according to the recommendations of Avnimelech (2009) and Boyd et al. (2016) and there was no need to supply the system with an inorganic carbon source, more precisely some type of limestone (calcitic, dolomitic or magnesian) to keep alkalinity and stabilize the pH. Besides, the alkalinity levels also increased with higher salinity levels in accordance with pH values (table 1).

Total ammoniac nitrogen or total ammonia is the result of the sum of non-ionized ammonia ($NH_3$), also called toxic ammonia, and ionized ammonia ($NH_4^+$). Only the 6 g/L salinity treatment (table 1) showed an average value below the maximum threshold recommended by El-Sayed (2006). However, toxic ammonia showed average

values for all treatmens below the tolerated threshold recommended by El-Sayed (2006) and El-Shafai et al. (2004), which is equal to 0.1 mg/L (table 1). This occurred because toxic ammonia is calculated from total ammonia, pH and temperature. The increase in pH due to treatments was not enough to promote the production of non-ionizable ammonia, to the detriment of ionizable ammonia. The temperature did not vary between treatments and did not influence the dissociation of ammonia. In addition to pH and temperature, salinity also influences the production of non-ionized ammonia, however, the increase in salinity also did not result in a measurable increase in the non-ionizable fraction of total ammonia. Despite the total ammonia being above the recommended threshold, the variables that influence the dissociation from total ammonia did not favor the formation of non-ionized ammonia, which is the one that really impacts the reduction in performance and mortality.

Nitrite is an intermediate inorganic compound for nitrification of ammonia and eight times less toxic than it (El-Sayed, 2006). Nitrite acts by binding to branchial receptors, which in turn are also chloride ion ($Cl^-$) receptors, thus competing at the receptor level. When bound to these receptors, the nitrite diffuses through the bloodstream and reacts with the hemoglobin present in the erythrocytes due to the affinity for the heme group, which consists of an iron atom contained in the center of a large heterocyclic organic ring called protoporphyrin IX. With this reaction, nitrite oxidizes hemoglobin, forming methemoglobin, a molecule that, unlike hemoglobin, has low affinity with molecular oxygen and inability to transport it from the blood to the tissues that demand $O_2$. Due to competition for receptors in the gills, the amount of nitrite that is actively transported into the bloodstream is influenced by the concentration of chloride ions in the culture water, which in turn is manipulated by the addition of sodium chloride (Yanbo et al., 2006). The accumulation of nitrite due to nitrification can be observed in BFT (Burford and Longmore, 2001; Hari et al., 2006; Azim and Little, 2008; Nootong et al., 2011; Widanarni Ekasari and Maryam, 2012; Luo et al., 2014; Alves et al., 2017; Alvarenga et al., 2018). This is due to the initial accumulation of chemoautotrophic bacteria and the insufficient water exchanges recommended for this type of system to reduce nitrite to lower levels (Ebeling et al., 2006; Hargreaves et al., 2006). In this experiment (table 1) nitrite concentration increased with higher salinity levels. In addition, the higher peaks of nitrite during the experiment (figure 1), despite the mature biofloc used to fill the tanks, were observed in the higher salinity concentrations (having the 8 g/L level, two high peaks).

The reduction in bacterial activity, mainly the activity of nitrifiers, due to an increase in water salinity, was pointed out by Alvarenga et al. (2018) as one of the reasons for the occurrence of higher peaks of nitrite and the increase in the average of this nitrogenous compound in the water. The results of pH, alkalinity and nitrite concentration can suggest that the increase in salinity inhibits the activity and/or growth of bacteria. The bacteria that grew in BFT were possibly adapted to a low salinity, since the experiment began in freshwater (and the floc matured too). The survival and growth of these bacteria may be impaired by variations in salinity, since salt ions exert high osmotic pressure on these microorganisms, and most of the freshwater-based microbial populations are unable to survive at these high osmotic pressures and either die or become dormant under these conditions (Altendorf et al., 2013; Wood, 2015). Thus, these results can indicate that nitrifiers and/or heterotrophic bacteria were possibly inhibited, at least partly, in the salt concentrations evaluated.

Nitrate is the final inorganic compound for ammonia nitrification. It is five hundred times less toxic than ammonia and 60 times less toxic than nitrite, according to Monsees et al. (2017). Also, based on the same authors, the toxicity threshold is 500 mg/L for Nile tilapia. The values of this variable remained within the recommended in the experiment. The average values of the first analysis were similar (table 1) and all averages at the second collection were different ($p < 0.05$) from the first ones, which shows that there was an increase due to the production by nitrifying bacteria from the produced nitrite. However, at the end of the experiment, the nitrate reduced with the increase of the salinity, corroborating with the nitrite results and the possibility of reduction of activity of nitrifiers.

Salinity is due to the presence of dissolved salts that represent 60 of the 92 basic chemical elements of nature. Salinity is a determining factor in controlling fish growth (Riley, 1965; Boeuf and Payan, 2001). Osmoregulation can lead to greater energy expenditure in the regulation of fish body fluids (10 to 50% of the energy balance) (Boeuf and Payan, 2001), which can have negative consequences in relation to oxygen consumption, food and hormonal regulation, directly interfering in the performance of cultivated species (Souza et al., 2019). According to Grau et al. (1994), fish have prolactin cells, which are directly osmosensitive. They also have, according to Laurent and Dunel-Erb (1984), pseudobranchial chemoreceptors that provide information about the salinity of the water. Osmoregulation is fundamental for the survival and performance of fish, not only due to the regulation of body fluids, but also for the production of mucus in the skin, which acts, physiologically, as an immunologically

active barrier in the protection against opportunistic and pathogenic microorganisms present in the environment of cultivation.

Several studies have shown that Nile tilapia shows better performance and growth at moderate salinities of approximately 5 to 12 g/L (Suresh and Lin, 1992; Likongwe et al., 1996; Kamal and Mair, 2005; Fridman et al., 2012), for growth phases after larviculture. In an experiment carried out in the same laboratory as the present study, Alvarenga et al. (2018) demonstrated that animals from 1 to 60 g of live weight, that is, in a phase sequential to the larviculture phase, had a superior performance in a salinity range of 4 to 8 g/L. In the present study, a superior performance was observed in lower salinities, close to zero, which leads us to interpret that younger animal, in the larval phase, do not tolerate salinities in which juveniles and adults present satisfactory performance for production, even adopting the adaptation period recommended by Lemarie et al. (2004).

Feed consumption (table 2) can be described as the difference between the ration offered and the leftovers. However, in the present experiment, the leftover was not measured, because the feed used was powder and this makes it impossible to measure the fraction rejected by the animals. The reduction in consumption according to the increase of salinity occurred because during the experiment a correction was made to the feeding rate as a function of mortality, apparently observed over the weeks, which allowed a readjustment of the quantity offered, necessary and inherent to the treatments.

Biomass is an important variable because it reflects the performance of animals as a group, or stock. It is a direct reflection of weight gain and survival. As there was a difference in the survival rate, due to the increase in salinity, this directly interfered with the final biomass, reducing it in treatments with higher mortality, since there was no difference in the mean final weight between treatments. In an experiment carried out in the same laboratory, with animals from 1 to 60 g, the best salinity found in the regression analysis to optimize biomass was around 6 g/L (Alvarenga et al., 2018).

Feed conversion ratio is a measure of productivity or growth performance that may be calculated from the ratio of feed consumption to biomass in case the sampling unit is the cultivation tank. It directly impacts the economic performance of any animal production activity. In the experiment carried out, the incorporation of sodium chloride in the cultivated water worsened the performance of this growth performance variable (table 2), because larvae increased the energy expenditure to perform osmoregulation, since in higher concentrations of salt, they tend to lose water to the environment. Alvarenga et al. (2018) found higher feed conversion ratio values at the highest

experimental salinities, in this case, 16 g/L for animals from 1 to 60 g. This shows the adaptive capacity to salinized water acquired by *O. niloticus* with weight gain and change in larvae stage to juvenile and adult stage.

The mean final weight did not showed difference between treatments. This was because in the treatments that presented higher mortality, there was a compensation in the weight gain of the survivors due to the decrease in the density of animals.

In an experiment carried out by Luz et al. (2013), in a clear water system, a statistically equal survival rate was observed between treatments 0 and 2 g/L, with a mean above 94%. For the 4 g/L treatment, a rate of 43% was found, statistically different from treatments 0 and 2 g/L. For the 6 g/L treatment, there was 100% mortality after ten days of the experiment. It should be noted that in the experiment by Luz et al. (2013), the adaptation recommended by Lemarie et al. (2004) was not applied, which severely compromised the survival of the stocked animals. However, it can be noted, similarly to what occurred in the present study, a superior survival in lower salinities. The high peaks of nitrite in the middle and at the last week of the experiment may have contributed to the mortality together with the increase in salinity per se.

According to Silva (2017), in hormonal treatments for 28 days it is possible to obtain high rates of masculinization in *O. niloticus*, equal to or greater than 94%, when using 17-α-methyltestosterone in the concentration of 60 mg/kg with food frequency minimum of 5 times a day for biofloc systems. In the present experiment, similar rates of masculinization were obtained, with no difference between treatments (table 2). However the results showed a tendency of reduction of the masculinization rate with the increase of the salinity (p-value = 0.0595), which suggests that the increase of salt in the water of the cultivation tanks might interfere in the masculinization protocol used in BFT.

In conclusion, the use of the salinized water in the masculinization period of the Nile tilapia larvae cannot be recommended in BFT. Although for Nile tilapia juveniles, bigger than 1 g, salinity close to 6 g/L promote much better growth by the reduction of the nitrite peaks toxicity (Alvarenga et al., 2018), for larvae the salinity close to 0 g/L promotes better survival, food conversion ratio and tends to guarantee higher masculinization rate.

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**Table 1.** Means, coefficient of variation (CV) and linear regressions of water quality variables of masculinization of Nile tilapia larvae reared at different salinities in BFT

| Variables | Salinity (g/L) | | | | | | | CV | Linear models; $R^2$; (p-value) |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | 0 | 2 | 4 | 6 | 8 | 10 | 12 | | |
| Temperature (ºC)* | 27.17 | 27.03 | 26.97 | 27.01 | 27.13 | 27.34 | 27.51 | 1.16 | (0.0697) |
| DO (mg/L)* | 7.16 | 7.37 | 7.44 | 7.46 | 7.41 | 7.32 | 7.11 | 2.58 | (0.6420) |
| pH | 8.06 | 7.76 | 7.86 | 8.02 | 7.95 | 8.16 | 8.27 | 1.3 | $Y = 7.85 + 0.03x$; $R^2 = 0.35$; (0.0045) |
| $CaCO_3$ (mg of $CaCO_3$/L) | 82.5 | 75.83 | 87.5 | 90.83 | 102.5 | 98.33 | 106.67 | 13.0 | $Y = 77.83 + 2.37x$; $R^2 = 0.45$; (0.0009) |
| SS (mL/L)* | 8.37 | 4.61 | 5.45 | 7.83 | 7.09 | 6.27 | 7.64 | 41.24 | (0.7435) |
| TSS (mg/L)* | 202.92 | 216.25 | 267.92 | 243.33 | 218.33 | 210.42 | 203.33 | 16.15 | (0.6157) |
| TAN (mg/L)* | 1.05 | 1.31 | 1.64 | 0.85 | 1.42 | 1.53 | 1.45 | 21.33 | (0.1957) |
| ToxTAN (µg/L)* | 37.98 | 46.90 | 60.22 | 31.18 | 53.96 | 58.14 | 56.14 | 22.12 | (0.0955) |
| Nitrite (mg/L) | 0.46 | 1.17 | 0.89 | 1.19 | 5.86 | 3.04 | 1.94 | 100.36 | $Y** = -0.48 + 0.14x$; $R^2 = 0.46$; (0.0007) |
| $NO_{3^-i}$ (mg/L)* | 144.45 | 149.15 | 186.91 | 168.01 | 169.86 | 159.45 | 164.75 | 20.69 | (0.5140) |
| $NO_{3^-f}$ (mg/L) | 272.16 | 305.46 | 285.80 | 219.51 | 261.90 | 239.09 | 241.83 | 14.14 | $Y = 287.35 - 4.42x$; $R^2 = 0.19$; (0.0499) |
| Salinity (g/L) | 0.22 | 2.06 | 4.02 | 5.9 | 7.72 | 9.46 | 11.61 | 1.13 | $Y = 0.21 + 0.94x$; $R^2 = 1.0$; (<0.0001) |

Reference values for tilapia culture: temperature = 27-32°C (El-Sayed, 2006); DO (Dissolved oxygen) > 4 mg/L (Wedemeyer, 1996); pH = 6-9 (Wedemeyer, 1996); $CaCO_3$ (Alkalinity) > 50 mg of CaCO3/ L (Avnimelech, 2009); SS (Settleable solids) < 100 ml/L (Avnimelech, 2009); TSS (Total suspended solid) < 1,000 mg/L (Avnimelech, 2009); TAN (total ammonia nitrogen) < 1 mg/L (El-Sayed, 2006); ToxTAN (toxic ammonia) <100 µg/L (El-Sayed, 2006); Nitrite < 8 mg/L (El-Sayed, 2006); $NO_{3^-i}$ (initial nitrate) and $NO_{3^-f}$ (final nitrate) < 500 mg/L (Monsees *et al.*, 2017).

* Linear regressions were not significant according to ANOVA (p > 0.05).

** Nitrite data was transformed in natural logarithm to properly normalize the values and then a linear regression model was fitted.

**Table 2.** Means, coefficient of variation (CV) and linear regressions of growth performance variables and masculinization rate of Nile tilapia larvae, during masculinization protocol at different salinities in BFT

| Variables | Salinity (g/L) | | | | | | | CV | Linear models; $R^2$; (p-value) |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 | 10 | 12 | | |
| FC (g) | 206.67 | 179.83 | 181.97 | 120.97 | 145.17 | 114.13 | 118.43 | 20.87 | $Y = 198.83 - 7.73x$; $R^2 = 0.52$; (0.0002) |
| $BM_f$ (g) | 219.95 | 194.13 | 174.36 | 88.21 | 88.13 | 72.34 | 62.16 | 27.8 | $Y = 213.43 - 14.16x$; $R^2 = 0.71$; (<0.0001) |
| FCR | 0.95 | 0.93 | 1.06 | 1.4 | 1.86 | 1.95 | 1.69 | 33.66 | $Y. = 0.86 + 0.09x$; $R^2 = 0.42$; (0.015) |
| $BW_f$ (g)* | 0.74 | 0.76 | 0.69 | 0.73 | 0.83 | 0.68 | 0.40 | 29.95 | (0.1107) |
| Surv (%) | 98.78 | 84.44 | 84.44 | 41.33 | 46.67 | 32.67 | 67.11 | 33.79 | $Y = 90.38 - 4.22x$; $R^2 = 0.33$ |
| MR (%)* | 96.12 | 93.86 | 92.47 | 96.60 | 86.82 | 92.51 | 86.61 | 6.75 | $Y = 96.09 - 0.66x$; $R^2 = 0.17$; (0.0595) |

Feed consumption, FC (g); final biomass, $BM_f$ (g); feed conversion ratio, FCR; final body weight, $BW_f$ (g); and survival, Surv. (%); masculinization rate, MR (%).

* Linear regression was not significant according to ANOVA (p > 0.05).

## pH



## Nitrite concentration
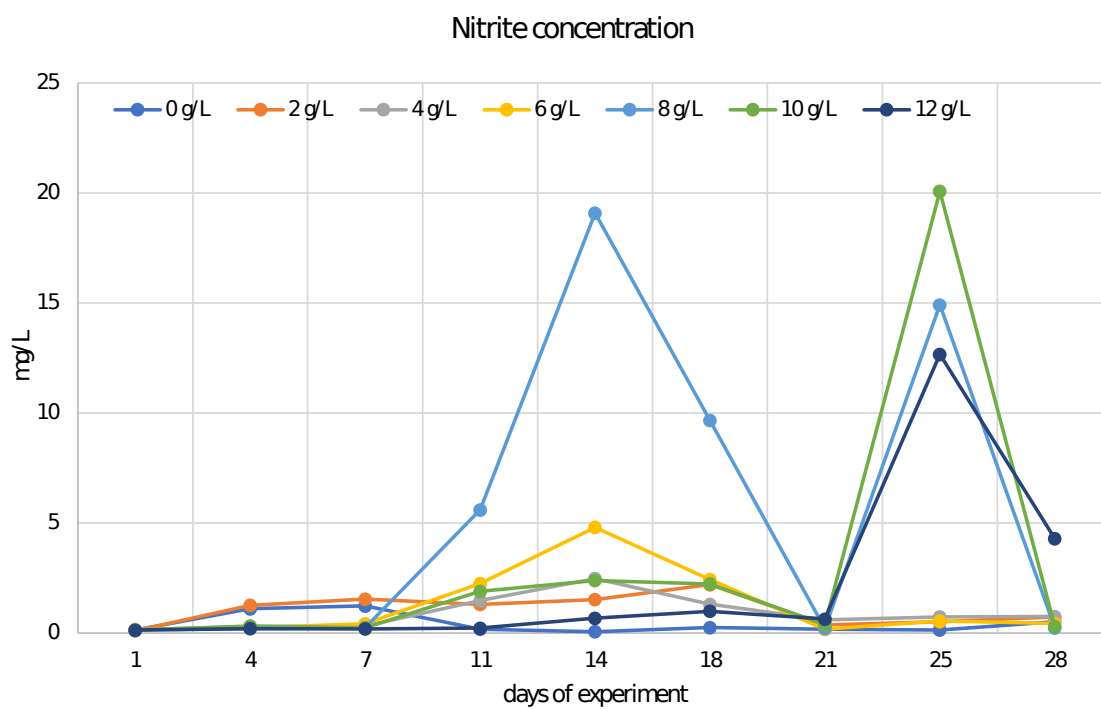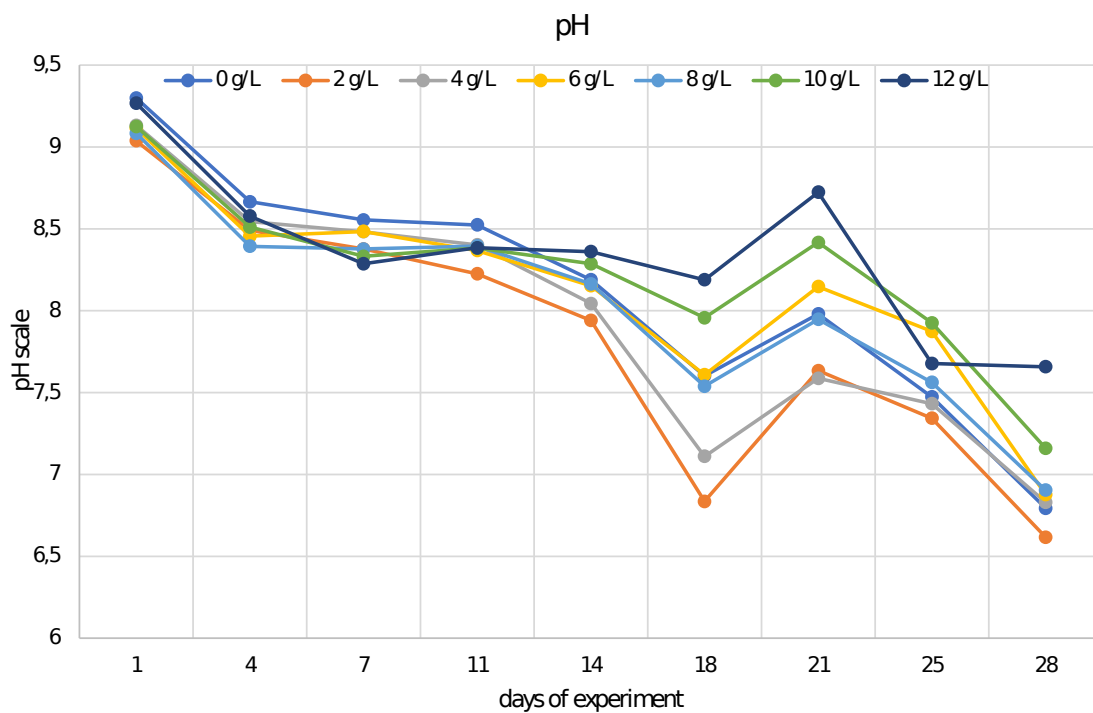


**Figure 1.** Means of pH and nitrite concentrations of seven different salinized water (0, 2, 4, 6, 8, 10 and 12 g/L) from tanks with biofloc, throughout masculinization experiment of Nile tilapia larvae

## 4. CONSIDERAÇÕES FINAIS

O aprimoramento do ambiente de cultivo visa maximizar o bem-estar associado ao maio desempenho zootécnico dos animais. A inclusão de sal nas fases iniciais do cultivo de tilápias é uma alternativa para melhorar a capacidade produtiva das formas jovens. Além disso, o sal diminui a toxicidade de compostos nitrogenados.

A produção de tilápias em sistema de bioflocos (BFT) é uma alternativa para uma maior intensividade de cultivo e menor consumo de água e custo operacional

O presente trabalho gerou a informações o produtor sobre as diferentes concentrações de sal em BFT para o cultivo de tilápias do Nilo.

É importante enfatizar que BFT necessita de monitoramento constante e mão de obra qualificada a fim de reduzir as possíveis mortalidades por problemas de qualidade de água.