DISSERTAÇÃO DE MESTRADO

NÍVEIS PROTEICOS E RELAÇÃO ENERGIA:PROTEÍNA NO DESEMPENHO ZOOTÉCNICO, ASPECTOS ENZIMÁTICOS, HEMATOLÓGICOS E PARASITOLÓGICOS EM BERÇÁRIO DE TILÁPIAS *Oreochromis niloticus* CULTIVADAS EM SISTEMAS DE BIOFLOCOS COM ÁGUA SALOBRA

EMERSON GIULIANI DURIGON

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Zootecnia, Área de Concentração Ciência e Produção Animal, da Universidade do Estado de Santa Catarina (UDESC), como requisito parcial para obtenção de grau de **Mestre em Zootecnia**

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como requisito parcial para obtenção do grau de
Mestre em Zootecnia

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A Deus.

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A todos os meus amigos irmãos:

MUITO OBRIGADO

RESUMO
Dissertação de Mestrado
Programa de Pós-Graduação em Zootecnia
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NÍVEIS PROTEICOS E RELAÇÃO ENERGIA:PROTEÍNA NO DESEMPENHO ZOOTÉCNICO, ASPECTOS ENZIMÁTICOS, HEMATOLÓGICOS E PARASITOLÓGICOS EM BERÇÁRIO DE TILÁPIAS O*reochromis niloticus* CULTIVADAS EM SISTEMAS DE BIOFLOCOS COM ÁGUA SALOBRA

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Chapecó, 24 de julho de 2018

A necessidade de se obter maiores produtividades em ambientes controlados tem feito com que o sistema de bioflocos cresça nos últimos anos, sendo a tilápia o peixe mais empregado neste sistema. O objetivo deste estudo foi avaliar níveis proteicos e relação energia:proteína, 3000, 3150 e 3300 kcal de ED/kg (Energia digestível) e 22, 26 e 30% de PD (Proteína digestível) no perfil de microrganismos no sistemas de bioflocos, desempenho zootécnico, composição bromatológica, parâmetros hematológicos, parasitologia e enzimas digestiveis de tilápias *(Oreochromis niloticus)* criadas em 10 ppm de salinidade. Foram utilizados 480 alevinos de tilápias (linhagem GIFT com 1,25 ± 0,15g de peso inicial) em 32 caixas com volume útil de 75L alimentados 3 vezes ao dia. Foi utilizada uma relação Carbono/Nitrogenio de 15/L sendo utilizado como fonte de carbono o melaço de cana de açúcar. Os resultados indicaram que o peso final, taxa de crescimento especifico e fator de condição foram menores para o nível de 22%, mas melhores para os níveis 26 e 30% de PD. Com o aumento de proteína houve aumento da proteína na carcaça. O teor de lipídeos aumentou conforme aumentou o nível de energia, tendo uma maior deposição de lipídeos na carcaça. Não houve diferença para a concentração de hemoglobina e o numero de leucócitos. No entanto, foi observado alterações no volume corpuscular médio (VCM) e numero de eritrócitos. Os níveis de pepsina aumentaram conforme o aumento da proteína na dieta. Já para tripsina, o tratamento com 30 % PD foi maior que os demais (22 e 26% PD); enquanto que para quimiotripsina o tratamento com 22% foi maior comparado aos demais. Foram encontrados monogenea na grande maioria dos animais amostrados, sendo que não houve efeito das dietas sobre a presença de parasitos nas tilápias. O grau de infestação foi baixo e visualmente não afetou o desempenho dos peixes. Em relação ao perfil da comunidade planctonica destaca-se um aumento do número de dinoflagelados e rotíferos ao longo do tempo e um decréscimo do número de microalgas. Com base nos resultados recomenda-se dietas contendo 26% de PD e 3000 kcal de ED para alevinos de tilápias criados em sistema de bioflocos em água salobra pois estes apresentaram melhores resultados de crescimento comparado ao 22% PD e foram semelhantes ao 30% PD, sendo que com o nível de 26% PD não houve prejuízo nos parâmetros sanguíneos, parazitológico, bromatológico e enzimático.

Palavras-chave: Biofloco, Nutrição, *Oreochromis niloticus,* Saude, Sanidade.

ABSTRACT
Master's Dissertation
Programa de Pós-Graduação em Zootecnia
Universidade do Estado de Santa Catarina

PROTEIN LEVELS AND ENERGY RELATIONSHIPS: PROTEIN IN ZOOTECHNICAL PERFORMANCE, ENZYMATIC, HEMATOLOGICAL AND PARASITOLOGICAL ASPECTS IN NUTRITIONAL TILAPS, Oreochromis nilóticus CULTIVATED IN BIOFLOCKS WITH SALOBRA WATER

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ADVISER: Mauricio Gustavo Coelho Emerenciano
Chapecó, 24 july 2018

The need to obtain greater productivity in controlled environments has caused the growth in the biofloc system in recent years; tilapia is the most used fish in this system. The objective of this study was to evaluate levels of protein and energy ratio: protein, 3000, 3150 and 3300 kcal ED / kg (digestible energy) and 22, 26 and 30% PD (digestible protein) in the profile of microorganisms in biofloc system, zootechnical performance, bromatological composition, haematological parameters, parasitology and digestive enzymes of tilapias (Oreochromis niloticus) grown at 10 ppm of salinity. A total of 480 tilapia fingerlings (GIFT strain with 1.25 ± 0.15 g of initial weight) were used in 32 boxes with a useful volume of 75L fed 3 times a day. A Carbon / Nitrogen ratio of 15 / L was used and sugar cane molasses was used as carbon source. Results indicated that the final weight, specific growth rate and condition factor were lower at the 22% level, but it is better at levels 26 and 30% of PD. With the increase of protein, there was an increase of protein in the carcass. The lipid content increased as the energy level increased, with a higher deposition of lipids in the carcass. There was no difference in hemoglobin concentration and number of leukocytes. However, changes in mean corpuscular volume (VCM) and number of erythrocytes were observed. Pepsin levels increased as protein increased in the diet. As for trypsin, the treatment with 30% PD was higher than the others (22 and 26% PD); whereas for chymotrypsin the treatment with 22% was higher compared to the others. Monogenea was found in the majority of sampled animals, and there was no effect of the diets on the presence of parasites in the tilapia. The degree of infestation was low and visually did not affect fish performance. In relation to the profile of the planktonic community, an increase in the number of dinoflagellates and rotifers over time and a decrease in the number of microalgae are noted. Based on the results, it is recommended diets containing 26% PD and 3000 kcal ED for tilapia created in brackish water biofloc systems because they presented better growth results compared to 22% PD and they were similar to 30% PD, and with the level of 26% PD there was no loss in blood, parazitological, bromatological and enzymatic parameters.

Keywords: Biofloco, Nutrition, *Oreochromis niloticus*, Health, Sanity.

LISTA DE FIGURAS

CAPÍTULO I – REVISÃO DA LITERATURA

CAPÍTULO II – MANUSCRITO 1

LISTA DE TABELA

CAPÍTULO II – MANUSCRITO 1

CAPÍTULO II – MANUSCRITO 2

SUMÁRIO

1. REVISÃO BIBLIOGRÁFICA

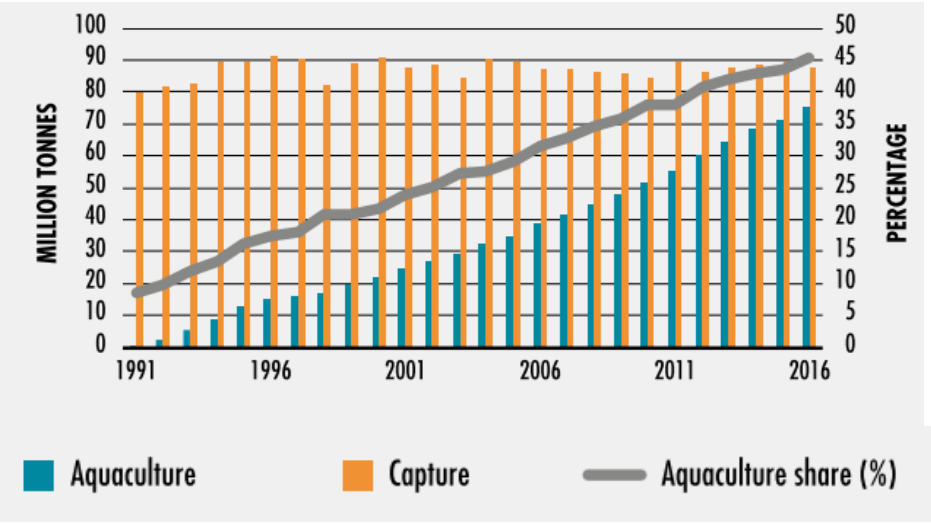
1.1. INTRODUÇÃO

1.1.1 Aquicultura

A aquicultura é uma das atividades do agronegócio que mais tem mostrado crescimento nos últimos anos e tem se mostrado como uma alternativa para atender à crescente demanda por alimentos de origem aquática (FAO, 2018). Segundo estimativas recentes a população mundial deve chegar a 9 bilhões de pessoas em 2050 (Carmo, 2007). Atrelados a esta explosão demográfica está a necessidade de comer bem e melhor. Neste sentido, a Organização Mundial da Saúde - OMS recomenda um consumo per capta de no mínimo 12kg de pescado por ano, no entando muitas regiões ao redor do globo ainda não atingiram estas cifras (WHO, 2007)

A estagnação da pesca mundial já não é novidade (Figura 1). Os estoques de peixes nos oceanos, rios e lagos vem diminuindo em todos os continentes e a aquicultura desponta como grande fornecedora mundial de proteína de altíssima qualidade (FAO, 2018). A aquicultura fornece praticamente a metade de todo pescado destinado ao consumo humano, aproximadamente 45% (FAO, 2018).

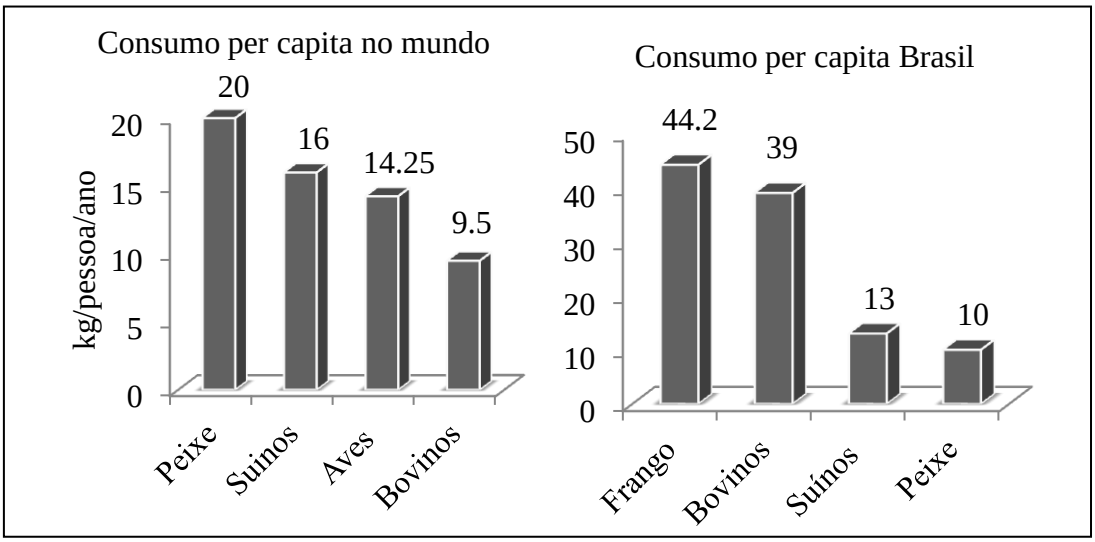
Figura 1: Evolução da pesca e aquicultura mundial.



Fonte; FAO 2018

O Brasil se destaca por ser um dos países com maior quantidade de área alagada passível para o uso da aquicultura, alem de ter uma costa marítima que pode ser explorada para a produção. Mesmo assim o consumo de peixe per capta no Brasil em 2015 é relativamente baixo, cerca de 10 kg/hab/ano (IBGE, 2015), perdendo para as outras três proteínas de origem animal (bovinos, frango, suino), com um consumo per capta de 44, 39 e 13 kg/hab/ano, respectivamente (Figura 2). No entanto, essa realidade não se reflete quando comparamos o consumo de proteína animal em todo o mundo. Pois o pescado lidera o ranking de consumo per capta que passou de 18,1 kg em 2009 para 20,2 kg em 2015(FAO, 2018).

Figura 2: Consumo per capto de proteína de origem animal no Brasil e no mundo.



Fonte; Elaborado pelo autor

O consumo de pescado está diretamente relacionado com a cultura de cada país. Além disso, o poder aquisitivo tem demostrado grande influência sobre o consumo desta proteína. Em regiões em desenvolvimento o consumo passou de 5,2 kg em 1961 para 18,8 kg em 2013, já em países com menor renda o consumo foi de 3,5 kg para 7,6 kg neste mesmo período (FAO, 2016). Em relação a América Latina, o Brasil terá o maior crescimento aquícola (104%) até 2025, superando países como México (54,2%) e Argentina (53,9%) (FAO, 2016).

A base desse crescimente, de acordo com o (IBGE, 2015), é o volume produzido pela piscicultura brasileira que representa quase 70% de toda a aquicultura nacional.

Este segmentou apresentou um cresccmento de 8% em 2017 comparado a 2016, sendo que os estados quem mais produziram em 2017 foram Paraná, Rondônia e São Paulo, respectivamente. O sul foi a região com maior produção (Figura 3). Adicionalmente, é estimado que a atividade movimenta cerca de R$ 5 bilhões ao ano, gerando 3,5 milhões de empregos diretos e indiretos (ACEB, 2014)

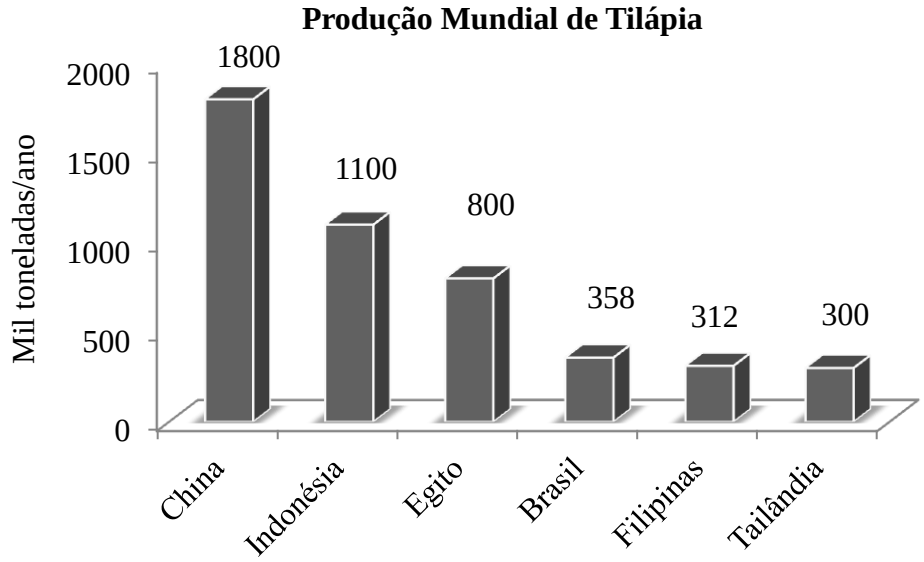
Figura 3: Dados da produção Brasileira por estado e regiões em 2017

Fonte; http://www.aquaculturebrasil.com/2018/02/19/peixe-br-lanca-o-anuario-da-piscicultura-2018/

1.1.2 Tilápia

Nativas da África, as tilápias possuem uma grande distribuição geográfica, estando presentes em todos os continentes, sendo que a China representa 45% da produção total desta espécie. O Brasil é o 4º maior produtor do mundo (Baptista et al., 2018). No Brasil é a espécie mais produzida, com um volume anual de 357.639 toneladas mil toneladas em 2017. Isso representa 51,7 % da produção nacional, seguido pelos peixes redondos tambaqui, pacu, pirapitinga e híbridos, que representam 43,7% da produção aquicola nacional (Baptista et al., 2018).

Figura 4: Principais países produtores da tilápia no mundo



Fonte; Elaborado pelo autor

Alguns fatores tem impulsionado o crescimento da produção de tilápias, tais como a fácil reprodução e adaptação ao ambiente de cativeiro, ótimo crescimento, rusticidade e excelente adaptação aos mais diversos sistemas de produção (Fitzsimmons and Alanis, 2011). Peixe de carne branca, textura firme e sabor delicado (Simões et al., 2007). Outra vantagem é o filé não apresentar espinhos em forma de “Y”, facilitando a indústria de filetagem (Meurer et al., 2003). O melhoramento genético, tolerância a uma ampla condições ambientais (temperatura, salinidade, baixo oxigênio dissolvido, etc.), maior resistência ao estresse e às doenças, também tem impulsionada a sua criação (Kubitza, 2011). No Brasil, outro fator pode contribuir na expanção da tilapicultura é a liberação

da produção em estados com grande potencial de produção como Tocantins e Mato Grosso, isso mostra que a participação da espécie na produção brasileira de pescado deve crescer ainda mais no futuro.

1.1.3 Proteínas em dietas para tilápia

A determinação da exigência nutricional para cada espécie é muito difícil, pois depende da fase, sistema de produção, hábito alimentar, entre outros fatores (Bicudo et al., 2010).

Estudos avaliando níveis proteicos para tilápias em sistema de recirculação com água clara já estão bem avançados e muitos autores avaliaram tais níveis aplicando o conceito de proteína ideal para diminuir o teor de PB com a adição de alguns aminoácidos essenciais (Furuya, 2010). Riguetti et al. (2011) incluindo lisina, arginina, metionina e treonina nas dietas e concluiu que é possível reduzir de 26,74 para 24,53% a proteína digestível em dietas para a tilápia do Nilo na fase de 100 a 500 g. Furuya et al., (2005) usando estes mesmos aminoácido em fase preliminar (5 a 125g) concluiu que é possível a redução de 30 para 27,5% de proteína digestível em sistemas de águas-claras. Fazendo um contraste com o passado, trabalhos clássicos como o de Furuya et al., (2000) testando níveis de proteína bruta para alevinos revertidos de tilápias de 0,5 a 13g conclui que para o máximo desempenho destes alevinos era necessário níveis de 32% de PB.

No entanto, o sistema de produção pode interferir diretamente na exigência do animal. Ferri et al., (2016) testando níveis de PB entre 28 e 36% para tilápias de 6 a 30g em sistema de bioflocos mostrou que o nível de 28% de PB pode mostrar desempenho satisfatórios. No entanto neste sistema de produção ainda são nescesário mais pesquisas para elucidar a real exigência de proteína e a relação energia/proteína para alevinos de tilápia.

1.1.4 Fase de berçário

Uma das fases considerada mais crítica para a produção de tilápia é a obtenção de juvenis de boa qualidade para iniciar o processo de engorda (Ayroza et al., 2011). Neste contexto, o valor de juvenis de maior tamanho, que seriam mais resistentes para estocagem, é muito elevado devido principalmente a baixa oferta e falta de tecnologias que viabilizem a produção dos mesmos em maiores escalas. Assim, a opção que resta ao produtor é adquirir o alevino de menor tamanho e fazer a primeira fase de crescimento em sua propriedade, o que aumenta o manejo e os custos de produção (Leonardo et al., 2009).

Neste sentido a fase de berçário é caracterizada como o período após a alevinagen e antes da engorda onde os peixes ficam em um ambiente mais reduzido e com maior controle de alimentação e contra os predadores, principalmente pássaros. Sendo assim, quando os peixes forem povoados em viveiro maiores serão mais resistente a doenças e predadores, possibilitando também diminuir o ciclo de produção e uma maior uniformidade no lote. (Leonardo et al., 2009).

Figura 5; Tilápias com 2 à 3g, peso onde seria recomendado a utilização do berçario

Fonte ; http://www.agenciasebrae.com.br/sites/asn/uf/NA/estudo-potiguar-aponta-melhor-sistema-para-criacao-de-tilapia,35ef8105b380d410VgnVCM2000003c74010aRCRD#prettyPhoto/0/

1.1.5 Biofloco

Grande parte da produção brasileira provém do sistemas semi-intensivos, que necessitam de uma renovação contínua de água. Porém é de suma importância a aplicação de novas tecnologias que intensifiquem os cultivos, maximizem a utilização de água e nutrientes, além de minimizar os impactos ambientais e melhorarem os índices produtivos (Hu et al., 2015).

Neste contexto, surge a tecnologia de bioflocos (CRAB et al., 2012, WASIELESKY et al., 2013), um sistema também conhecido como "biofloc technology" (ou BFT na sua sigla em inglês), considerado a "revolução azul", pois preconiza uma máxima produção com baixos impactos ambientais, mínima geração de efluentes e um crescimento sinergético entre peixes/camarões e a comunidade de microrganismos (EMERENCIANO et al., 2013). Estas comunidades microbianas ajudam no controle das variáveis de qualidade de água, especialmente dos compostos nitrogenados (Avnimelech and Kochba, 2009), no suprimento de nutrientes e no controle de patógenos (Emerenciano et al., 2017). Estes agregados (chamada de bioflocos) é composto por restos de ração, fezes, material inorgânico em suspensão, além de uma diversa comunidade microbiana tais como bactérias heterotróficas, fitoplâncton, protozoários, nematóides, rotíferos, copépodos, entre outros, mantidos em suspensão na coluna d'água por constante movimento e aeração (CRAB et al., 2007, RAY et al., 2010).

Dentre as diversas vantagens deste sistema se destacam a biosseguridade (cultivos mais fechados e controlados), produção de grandes quantidades de biomassa de pescado em um pequeno espaço e o aspecto nutricional onde os flocos também servem de alimento para os animais cultivados (CRAB et al., 2007, AVNIMELECH; KOCHBA, 2009). Este alimento natural pode suprir em algumas espécies boa parte da demanda proteica, como relatado por Azim e Little, (2008) com uma redução de 11% nos níveis de proteína bruta nas dietas de juvenis de tilápias.

Este sistema depende de uma relação equilibrada de carbono e nitrogênio na água para o seu correto funcionamento (Crab et al., 2012), estimulando o crescimento dos organismos microbianos no sistema promove a absorção de compostos nitrogenados e transformação de resíduos em proteína microbiana (AZIM; LITTLE, 2008, EMERENCIANO et al., 2011).

Lima et al. (2015), testando três densidades de estocagem de tilápia em sistema de biofloco (15, 30 e 45 peixes/m3) com peso médio de 123,0±0,6g, concluiu que a densidade de estocagem de 45 peixes/m3 apresentou a melhor resposta, uma vez que foi

possível atingir a maior produtividade e sobrevivência de 91%. Já Brol et al., (2017) testando 400 e 800 tilápias/m³ com 3,06 ± 0,2g e comparando duas linhagens *(O. niloticus)* linhagem GIFT e linhagem vermelha encontrou bons resultados comparado com outros trabalhos destas mesmas espécies e concluiu que a maior densidade 800/m³ traz melhora nos parâmetros zootécnicos. Já Ekasari et al., (2015) testando a reprodução e a larvicultura de tilápias em sistema de biofloco comparado com água clara recomendam o uso de biofloco em tanques de reprodutores e durante a larvicultura de tilápia do Nilo, para melhorar a qualidade e o desempenho das larvas produzidas.

Figura 6; Criação de tilápia em sistema de bioflocos

Fonte; https://www.youtube.com/watch?v=xOIG7vG_xNI

1.2. OBJETIVOS

1.2.1Objetivo geral

O objetivo deste estudo foi avaliar se diferentes níveis proteicos e relação energia:proteína tem efeito possitivo no desempenho zootécnico, composição bromatológica, aspectos enzimáticos, hematológicos e parasitológicos na fase de berçário de tilápias (linhagem GIFT) cultivadas em sistemas de bioflocos em água salobra.

1.2.2 Objetivos específicos

- Avaliar se os níveis proteicos de 22, 26 e 30% de PD e energéticos de 3000, 3150 e 3300 kcal de ED/kg em água de cultivo com salinidade 10.
- Monitorar ao longo do período experimental o perfil de microrganismos
- Avaliar se as dietas afetam a composição bromatológica do biofloco (biomassa microbiana);
- Avaliar se dietas com diferentes níveis proteicos e energéticos em sistema de bioflocos afeta o hemograma dos peixes assim como a carga parasitória;
- Determinar se as dietas afetam as atividades enzimáticas no estômago (pepsina) e intestino (tripsina, quimiotripsina e lipáse).

1.3. HIPOTESE

Usar dietas com 26 % de PD para tilápias sem prejuízos nos parâmetros de desempenho zootécnico e sem alterações prejudiciais a saúde dos peixes nos parâmetros sanguíneos bioquímicos, enzimáticos e bromatológicos, para que assim possa se usado esta dieta em cultivos de alevinos de tilápias em sistema de bioflocos.

2.CAPÍTULO II (MANUSCRITOS)

Os resultados desta dissertação são apresentados na forma de dois manuscritos com sua formatação de acordo com as orientações da revista Aquaculture ao qual seram submetido, sendo eles entitulados:

MANUSCRITO 1: Tilápia do Nilo *(Oreochromis niloticus)* alimentadas com diferentes níveis de proteína e energia digestível em sistemas de bioflocos com água salobra

MANUSCRITO 2: Enzimas digestivas e parasitologia de tilápias criadas em sistema de bioflocos com 10 ppm de salinidade e alimentadas com diferentes níveis de proteína e energia

2.1 – MANUSCRITO I

Berçário de tilápia do Nilo (Oreochromis niloticus) alimentadas com diferentes níveis de proteína e energia digestível em sistemas de bioflocos com água salobra

Emerson Giuliani Durigon, Giovanni Lemos de Mello, Tayna Sgnaulin, Gabriela Tomas Jerónimo, Michele Cristina Thomas e Maurício Gustavo Coelho Emerenciano

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com fator de impacto igual a 1,893.

Journal: Aquaculture (Elsevier)

Research Article:

Nile Tilapia (Oreochromis niloticus) fed with different levels of protein and digestible energy in biofloc systems with brackish water

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ABSTRACT: The objective of this study was to evaluate protein leves and energy relation : protein, 3000, 3150 and 3300 kcal of digestible energy (ED / kg) and 22, 26 and 30% of digestible protein (PD) in the parameters of zootechnical performance, carcass bromatological composition, hematological parameters and profile of microorganisms in the cultivation of tilapia (Oreochromis niloticus) grown in 10 ppm saline biofloc systems. A total of 480 tilapia fingerlings (GIFT strain with 1.25 ± 0.15 g initial weight) were used in 36 boxes with a useful volume of 75 liters fed 3 times a day. According to the results, the final weight, specific growth rate and condition factor were better for levels 26 and 30% of PD. As protein increase occurred in the diet, there was an increase in the protein in the carcass. In relation to the lipid content, the higher the energy level the greater the lipid deposition in the carcass. There was no difference in hemoglobin concentration and in leukocytes, which may show that fish were well immunologically. It was observed changes in mediun corpuscular volume (MCV) and number of erythrocytes, which may indicate a possible compensatory effect of body by reducing the number of erythrocytes. There was an increase in the number of dinoflagellates and rotifers as well as the decrease in the number of microalgae throughout the experimental period. Diets containing 26% PD and 3150 kcal ED are recommended for tilapia fingerlings raised in a biofloc system in saline water at 10 ppm.

KEYWORDS: BFT, Growth, Hematology, Nutrition.

INTRODUCTION

Aquaculture is one of the agribusiness activities that has shown the most growth in recent years and presents great potential to meet the growing demand for food by animal origin (FAO, 2016). Much of the production comes from the semi-intensive systems that need continuous water renewal, increasing the risks of introducing pathogens and adverse effects to the environments adjacent to the farms. New technologies are used to focus crop intensification with minimal water disposal. Among the new technologies is the biofloc system, which is a system characterized by minimum or zero water exchange, control of nitrogen compounds and formation of microbial aggregates that can serve as a live food 24 hours a day (Emerenciano et al. , 2017). In addition, it maximizes the use of water and nutrients, minimizing

environmental impacts (Wasielesky et al., 2006). In the BFT system by means of null or minimal water changes, the handling of C: N ratio and intense movement and aeration of the water, microbial aggregates suspended in the water column are formed (Crab et al., 2007). These aggregates or bioflocos are formed by an inert fraction (feed remnants, faeces and organic material) and another composed by heterotrophic bacteria, chemothiotrophic, phytoplankton, protozoa, nematodes, rotifers, copepods and other microorganisms. These bioflocos serve as rich food available 24 hours a day and help improve fish production indexes (Hu et al., 2015), and they may even supply a portion of the animal protein demand as reported by Azim and Little (2008).

Tilapia (Oreochromis niloticus) is a species that is well accepted by consumers mainly because it does not present intramuscular spines, besides being a white meat with firm texture (Simões et al., 2007). This species has rapid growth, rustica, it is being well adapted to intensive production systems, it also has an omnivorous feeding habit and it is a filtering agent, which contributes to its adaptation to intensive systems rich in natural food (Fitzsimmons, et al., 2011). Tilapia production is carried out in several systems and environments, including areas with moderate salinity (Likongwe et.al. 1996). Although domesticated and expanding, its cultivation still faces technological challenges, and the application of the nursery stage in bioflocos could increase the productive cycles per year and increase the control and biosafety in the initial phases since this stage of tilapia creation still faces some difficulties, sometimes with high levels of mortality and low growth.

In relation to the researches which used tilapia in a biofloc system, some studies were carried out with the use of different types of breeders (Ekasari et al., 2015), with different stocking densities (Brol et al., 2017; Haridas et al 2017; Rodrigues et al. , 2015), different protein levels (da Silva et al., 2018; Abdel-Tawwab et al., 2010), carbon-nitrogen ratio (Pérez-Fuentes et al., 2016) And other carbon sources (Zhang et al., 2006). However, there are no papers relating the energy ratio: protein to tilapia in a biofloc system in the early stages with low salinity. The objective of this study was to evaluate levels of protein and energy ratio: protein (3000, 3150 and 3300 kcal ED / kg and 22, 26 and 30% PD) in the zootechnical performance, hematological aspects and carcass composition in tilapia- Nile (Oreocrhomis niloticus, lineage GIFT) in the nursery stage in salinity 10 ppm.

MATERIAL AND METHODS

Experimental design

The experiment was realized at the Aquaculture Laboratory, in the State University of Santa Catarina (UDESC), campus from the Southern Higher Education Center (CERES), in Laguna, Santa Catarina, Brazil.

A total of 480 fingerlings tilapia of *Oreocrhomis niloticus*, GIFT lineage (1.25 ± 0.15 g initial weight) were populated in 36 experimental units (circular plastic boxes with a useful volume of 75L called "microcosmos") distributed in three benches (12 units each bench) in a completely randomized manner. The treatments were constituted with different combinations of digestible levels of PD (22, 26 and 30% PD) and different energy levels (3000, 3150 and 3300 kcal), totaling 9 treatments with four replicates (Table 1). Each bench contained a matrix tank (macrocosm), (circular plastic boxes with a useful volume of 900L each), connected to each other, and with two 200 W heaters per box to control water temperature.

Each matrix tank received an initial biofloc inoculum (300L) from previous BFT tilapia cultures, and it was completed to a volume of 700L with fresh water, then 300L of water with 35 ppm salinity was added, resulting in a final salinity of 10 ppm. For the formation and maintenance of the microbial community, a biomass of 2.5 kg.m-3 (juveniles of tilapia of approx. 70 g) was maintained in each matrix tank, fed with commercial feed (32% PB supplied 2x / day; : 00 and 18: 00h) and a C: N ratio of 15: 1 (Emerenciano et al 2017), where powder sugar cane molasses was used as an external carbon source.

Aiming for constant aeration and water movement, each experimental unit had central individual aeration by means of porous stone (20 mm in diameter and 30 mm in height) and, in each matrix tank, the aeration was composed by a ring of microperforated hose (70 cm perimeter) that was located centrally on the bottom of the tank. In order to maintain the same water quality and the same qualitative-quantitative profile of microorganisms in all experimental units, the water from the matrix tanks was pumped to the experimental units with gravity return. In addition, the matrix tanks (macrocosms) were interconnected to maintain the same water quality between the benches. Before the beginning of the experiment, the fingerlings (15 per tank) were acclimatized for one week with brackish water, which was gradually increasing until the

end of the fifth day; it reached a salinity of 10 ppm. The total experiment time, after acclimatization, was 42 days.

Food management and preparation of diets

The diets were formulated and manufactured in the Nutrition of Aquatic Organisms Laboratory (LANOA), from the State University of Santa Catarina. The formulations had established levels of digestible protein (% PD) and digestible energy (kcal of ED / kg), totaling nine treatments: 22: 3000, 22: 3150, 22: 3300, 26: 3000, 26: 3150, 26: 3300 , 30-3000, 30: 3150, 30-3300 which were isophosphoric and isocalcitic (Table 1). The protein and digestible energy values from ingredients were calculated based on Furuya (2010).

For the diet preparations, the ingredients were milled and sifted with 300 μm sieve, individually weighed, and they were mixed and mechanically homogenized. Afterwards, approximately 20% of water was added to the mixture and the pelletizing process was made by using a meat grinder (1/3 hp engine). The processed pellets were oven dried (55 ° C) for 72 hours and crushed to form particles with a particle size of 2 mm. Animals were fed according to the biomass of each experimental unit (8% in the first two weeks and, after, 5%) with a frequency of three times a day (8:00 a.m., 1:30 p.m. and 6:00 p.m.).

Analyzes of water physico-chemical parameters

Water quality parameters such as dissolved oxygen, temperature (YSI-55 oximeter, Yellow Springs Instruments, OH, USA) and pH (MS Tecnopon®-mPA-210, São Paulo-SP, Brazil) were monitored daily in the macrocosm and in each treatment. Ammonia (NH3-N), nitrite (NO2-N), nitrate (NO3-N), orthophosphate ($PO43^{-}$) and total alkalinity (CaCO3) were analyzed once a week (days 7, 14, 21, 28, 35, 42). Reading of nitrogenous concentration compounds was carried out with photocolorimeter (microprocessed AT 100P, ALFAKIT, Florianópolis, SC, Brazil). Total alkalinity was determined by using the volumetric titration method with commercial kit (ALFAKIT cod. 2058 and 2460).

Suspended bioflocs volume (sedimented solids) was measured daily using a 1000mL Imhoff cone, by sedimentation of one liter of water sample for 20 minutes, according to methodology described by Eaton et al. (1995).

Counting and identification of biofloc microorganisms

To characterize the composition of microorganisms present in the BFT system, water samples were collected during the six experimental weeks, and three samples of 50 ml (one in each macrocosm) were collected per week (days 7, 14, 21, 28, 35, 42), totaling 18 samples. It was added formalin (4%) and they were blushed with Bengal Rose for later counting and identification of organisms present. Counting and identification of the invertebrates were performed in cross-linked Petri dishes, using a stereoscopic magnifying glass (Ultralyt® LBP2-4). For the microorganisms, it was made two homogenized sub-samples of 1 mL each, and it was used Sedgewick-Rafter chambers (Azim and Little, 2008; Emerenciano et al., 2013), where 50 chamber spaces were counted for each sample.

Bromatological analyzes

The dry matter, crude protein and ash flake and fish content were analized according to the methodology proposed by AOAC (1999). To determine the lipid content, the Bligh and Dyer technique (1959) was used. For the bioflocos, the cultivation water (approx. 400 L from the three macrocosms) was decanted by approx. 30 minutes and the supernatant discarded. This operation was repeated several times until a material with the lowest amount of water was obtained, and then, with the remaining material dried (oven drying at 55 ° C for 72 hours or until reaching a stable dry weight) for analysis (crude protein, lipids and ashes).

Performance Parameters

In the end of the study, the following performance parameters were analyzed: standard length (cm), survival (%), final weight (g), productivity (kg / m3), condition factor, hepatosomatic index, apparent feed conversion, specific growth rate (% / day), carcass yield (%), protein efficiency rate and viscous-somatic index.

Hematological analyzes

At the end of the experiment, 10 fish per treatment were anesthetized with eugenol (1 mg.L-1) for hematological analysis. After biometry, the blood was collected by puncturing the caudal vessel, using syringes containing 10% EDTA. The percentage of hematocrit (Goldenfarb et al., 1971), hemoglobin concentration (Collier, 1944) and total erythrocyte count after dilution of 1: 200 in sodium chloride solution (Ranzani-Paiva et al., 2013). For total plasma protein analyzes (PPT), blood samples were separated into microtubes and centrifuged at 3000 RPM for 10 minutes and determined by colorimetric methodology (Analisa® kit).

For total leukocyte count and leukocyte differential, blood extensions were made on blades which were blushed with May-Grunwald / Giemsa (Rosenfeld, 1947), and the reading was performed by the indirect method (Ishikawa; et al 2008). Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (HCM) were also calculated.

Statistical analysis

After verifying the normality (Shapiro Wilk) and homoscedasticity (Levene) of the data, the values that did not present normal distribution were transformed before analyzed, but, for presentation in tables, the original data were maintained. The results were submitted to analysis of variance (two-way ANOVA); and subsequently significant differences between the treatments were detected by the Tukey test. All data were analyzed at 5% level of significance.

RESULTS

Water Quality

The parameters of water quality, oxygen, temperature and pH did not present significant variation among the treatments (Table 2). Variation of nitrogen compounds, orthophosphate, alkalinity, and biofloc volume throughout the experiment are shown in Figure 1. Ammonia and nitrate remained stable throughout the experiment, while nitrite presented an increase in the fourth week.

Zootechnical performance

There was no interaction (protein x energy) for the variables of zootechnical performance (Table 3 and 4). For the standard length (CP), the two lowest energy levels (3000 and 3150 kcal ED) presented higher values. At levels of 26% PD, CP was significantly higher compared to 22% ($P < 0.05$). IHS was higher in treatments containing 22% PD whereas FC was lower at the 22% level of PD (Table 3).

For the variables of final weight (PF), apparent feed conversion (CAA), survival (SOB), productivity (PRO) and specific growth rate (TCE), no significant difference was found between the energy tested levels ($P > 0, 05$). However, comparing protein levels, it was observed that for PF and TBI, there was a difference ($P < 0.05$) between 22% and 26 and 30% PD. TEP with the level of 30% PD differed from the others. The productivity (Kg / M3) was higher with the levels of 26 and 30 of PD (Table 4).

Bromatological composition

The dry and mineral matter contents were not influenced by the treatments (Table 5). However, for the PB content in the carcass there was an increase as the increase of PD in the diet, and lipid in the carcass increased as the ED increase in the diet (Table 5). For these two parameters (protein and fat) there was interaction between energy levels and proteins which were tested (Figure 2). At the 22% PD level, as the energy increase there was an increase in the lipid content in the carcass, the same was not observed at the PD 26 level where the level of 3300 kcal ED was equal to the others (Figure 2).

Haematological parameters

There was no difference in hemoglobin concentration, HCM, leukocytes and lymphocytes numbers (Table 6). For erythrocytes and VCM there was difference only when compared to energy levels, where the average with 3150 kcal ED differed from the average of 3000 kcal ED. There was an increase in the number of monocytes in fish fed diets containing 26% PD compared to 30% PD. The number of neutrophils was influenced by protein levels, where animals fed with 26% PD showed higher values

(Table 6). For hematocrits and PPT, there was interaction between protein and energy levels (Figure 3).

Characterization of microorganisms

During the six weeks, protozoa, nematodes, rotifers, copepods, dinoflagellates and microalgae were identified. The highest frequency groups are shown in Figure 4, where the number of rotifers and dinoflagellates can be observed over time ($p < 0.05$), while the protozoa showed a fall in the third week, with a significant increase in the sixth week. On the other hand, the microalgae had greater variation and they obtained a significant difference where there was a decrease between the first and sixth week of culture ($p < 0.05$).

DISCUSSION

Water quality

The water quality remained within the recommended for the species (El-Sayed, 2006), and similar to that found by Azim; Little, (2008) with O. *niloticus* in a biofloc system. The fact that there is no oscillation over the six weeks in the ammonia levels demonstrates an advantage of the biofloc system, since the microorganisms present in the water control this toxic compound for the fish (Kishida et al., 2008).

The alkalinity decreased in the sixth week compared to the fourth one, this can be explained by the significant increase in the flake volume after the third week. The consumption of calcium carbonate (CaCO3) by microorganisms to oxidize ammonia and convert it in microbial biomass may explain the decrease in alkalinity (Ebeling; Timmons; Bisogni, 2006). The increase of sedimentable solids that happened also after the second week was also found by Brol et al. (2017), it shows that after this, there is a higher production of flakes in the system, and the average over the six weeks did not exceed 30 mg.L-1 as recommended for tilapia cultivation (Avnimelech, 2007). It can be observed that, in the fourth week, there was a decrease in orthophosphate levels; this result was attributed to the addition of 400L of water in the system due to evaporation.

Zootechnical performance

Nowadays, studies that evatuate the energy and protein levels for tilapia in light-water systems show the protein requirement for tilapia is between 27 and 29% PD (Furuya et al., 2000; Junior et al., 2016). However, in the present study the final weight, specific growth rate and productivity had similar results between the levels of 26 and 30% of PD, but higher than 22%. This fact may be related to the presence of biofloc microorganisms that contribute to the nutrition of tilapia, as the flake present in this system contained 17.39 ± 0.20% PB, and 1.22 ± 0.09% of lipids.

The reduction of protein levels for tilapia in biofloc systems has also been demonstrated by Azim and Little (2008). However, the effect on energy levels has not yet been elucidated, since high energy levels may end up limiting the consumption of nutrients (Boscolo et al., 2005). Low energy levels can also cause serious production problems, since protein will be used as a source of energy, thus increasing feed conversion and ammonia excretion into the system (Pezzato et al., 2002). In this study, energy levels did not influence the zootechnical performance parameters which may have been due to the presence of microorganisms in the system that could be used as a source of energy or protein.

The hepatosomatic index (IHS) was influenced by the protein level, where the lowest level of protein had the highest IHS, same result found by Gallagher (1999). This can be explained due to a form of compensation, where due to a lower availability of protein, the body had to adapt by increasing the size of the liver to metabolize as much protein as possible, similar results were found by Santos et al., (2018) which evaluated protein levels for *Prochilodus argenteus*. The hepatosomatic index (IHS) was influenced by the level of protein, where the lowest level of protein had the highest IHS, same result found by Gallagher (1999). This can be explained, due to a form of compensation, where due to a lower availability of protein, the body had to adapt by increasing the size of the liver to metabolize as much protein as possible, similar results were found by Santos et al. (2018) evaluating protein levels for Prochilodus argenteus.

Protein levels also influenced FC, a comparative index, providing important information about the physiological state of the animals (Lima-Junior et al., 2002). However, no difference was observed between the levels of 26 and 30% of PD, which shows that with 26% of PD the animals are in good physiological condition. For protein efficiency, levels of 22 and 26 were similar ($P > 0.05$) and higher than 30% PD. This pattern shows that with lower levels, less protein waste occurs, avoinding loss to the

medium. It is important to note that feed frequency (Furuya et al., 2005) and the presence of "biofloc" natural food (Martínez-Córdova et al., 2015) may have contributed to this result.

Carcassess bromatology

The higher content of lipids in the fish carcasses which were fed with higher number of proteins resembles the results found by Bomfim et al. (2008). This fact can be explained by the greater amount of exced amino acids for catabolism, which may result in a higher caloric increment and a lower fraction of net energy deposited as body fat (Dabrowski and Guderley, 2002). A lower body fat content of fish which were fed with higher protein level (lower ED: PB ratio) was also observed in trout (*Oncorhinchus mykiss*) by Yamamoto et al. (2005). The results of this study are not in agreement with the ones finding by Meyer and Fracalossi (2005), who observed that body fat reduces with the increasing of crude protein in diet.

In addition, the level of 3300 ED presented higher fat content in the carcass in treatments 22 and 30% PD, since when there is excess energy (22: 3300) or protein excess (30: 3300), fish deposited higher content of fat. Protein when excess is broken and destined to gluconeogenesis, which can lead to greater accumulation of fat, since the greater amount of energy is also related to fat accumulation, production of adipose tissue (NRC, 2011). Meyer and Fracalossi (2005) observed that body fat was higher ($p < 0.05$) in fish which were fed with higher energy diet. Body fat was also higher for blue tilapia (*Oreochromis aureus*) that was fed with higher energy level (Wille et al., 2002). As well as surubins that were fed with increasing energy levels they presented an increased body ethereal extract (Martino et al., 2002).

However, it should be noted that the biofloc composition may also have interfered in the composition of the fish carcass, since this flake contained 1.22 ± 0.09 and 17.39 ± 0.20 of lipids and crude protein in the dry material, respectively. The lipid composition has already been studied by Azim and Little (2008), they showed that the biofloc contains a great quantity of polyunsaturated fatty acids (PUFAs) Omega 3 and 6, which is very good from a nutritional viewpoint in terms of tilapia feeding.

Hematology

This work showed that protein and energy levels did not interfere in erythrocytes hemoglobin. The changes in VCM and in number of erythrocytes suggest that there was a possible compensatory effect of the body by reducing the number of erythrocytes, and it caused the increase of VCM, so that it could reduce the damage caused by the reduction of red cells, allowing the transport of O2 in desirable levels to the issues. The higher presence of monocytes and neutrophils in the treatment with 26% PD may indicate an improvement in the defense system, since these cells are responsible for an immune response in the defense against a pathogen (Antunes et al., 2017).

For total plasma protein (PPT), there was interaction, where at 22 and 26% PD levels there was an increase in 3150 kcal ED kg-1 compared to 3000 kcal ED kg-1. This demonstrates that these protein levels may not meet the requirements of fish when using 3000 kcal ED kg-1, since PPT can be used as an amino acid source by cells (Wu, 2013). In addition, increased hematocrit at the lowest energy and protein level may have occurred due to increased oxygen transport and to a possible nutritional stress (Morales et al., 2005). However at the 3150 level of energy, it was observed an increase in hematocrit when compared to 30% PD with 22 and 26% PD, this fact can be explained due to a higher oxygen requirement of the organism to degrade proteins, similar results founded by (Camargo; Martins, 2005).

Profile of microorganisms

For the microorganisms, it was observed a variation over the weeks, it can be as result of the luminous intensity, predator / prey ratio (rotiferous and dinoflagellates / microalgae), competition for substrate with the bacteria and other organisms. This fact may also be related to fish consumption of microorganism (Emerenciano et al., 2013). The decrease of the microalgae may also be related to the lower light presence due to the greater turbidity of the water that was generated by the flakes. The turbidity in the BFT added the absence of light can have effect in the number of protozoa, since for these microorganisms the heterotrophic metabolism prevails. The higher abundance of protozoa in the biofloc system which was observed in this study corroborates to the studies by Azim and Little, (2008) and Emerenciano et al.; (2013).

The higher presence of rotifers from the fourth week may show that these microorganisms are more present in the intermediate phases of the biofloc, probably by the adjustment of the prey predator relation, as reported by (Brol et al., 2017). This

group of microorganisms has a fundamental role for the system, because it contributes to the fragmentation and formation of flake due to mucus excretion. It is important to mention that these groups of microorganisms are of great value for the efficiency of this activity since they contribute to improve CA, once some groups are considered as alternative food items for the cultivated organisms (Azim and Little, 2008; Emerenciano et al., 2007; Martínez-Córdova et al., 2015).

CONCLUSION

Based on the results of this study, it is concluded that the biofloc system is efficient in controlling water quality parameters, mainly for nitrogen compounds, which is fundamental for success in intensive production. There was an interaction among microorganisms present in the system, which contributed to the feeding of the tilapia, since for the parameters of zootechnical performance, levels of 26 and 30% PD had similar results. Also the levels of 26% PD and 3150 kcal ED had the best results for blood parameters. The diet compositions affected the bromatological parameters of the carcass. Based on these parameters, it is recommended to use 26% PD and 3150 kcal ED for tilapia fingerlings which are created in a biofloc system with 10 ppm salinity.

REFERENCES

Abdel-Tawwab, M., Ahmad, M.H., Khattab, Y.A.E., Shalaby, A.M.E., 2010. Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, Oreochromis niloticus (L.). Aquaculture 298, 267–274. doi:10.1016/j.aquaculture.2009.10.027

Antunes, B.D.M., Rossi, F.E., Inoue, D.S., Neto, J.C.R., Lir, F.S., 2017. Imunometabolismo e Exercício Físico: Uma nova fronteira do conhecimento. Motricidade 13, 85–98. doi:10.6063/motricidade.7941

AOAC, 1999. AOAC International, Official methods of analysis, 16th edn. ed. Washington.

Avnimelech, Y., 2007. Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. Aquaculture 264, 140–147. doi:10.1016/j.aquaculture.2006.11.025

Azim, M.E., Little, D.C., 2008. The biofloc technology (BFT) in indoor tanks: Water

quality, biofloc composition, and growth and welfare of Nile tilapia (Oreochromis niloticus). Aquaculture 283, 29–35. doi:10.1016/j.aquaculture.2008.06.036

Bligh, E., Dyer, W., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. 37, 911–917.

Bomfim, M.A.D., Lanna, E.A.T., Donzele, J.L., Abreu, M.L.T. de, Ribeiro, F.B., Quadros, M., 2008. Redução de proteína bruta com suplementação de aminoácidos, com base no conceito de proteína ideal, em rações para alevinos de tilápia-do-nilo. Rev. Bras. Zootec. 37, 1713–1720. doi:10.1590/S1516-35982008001000001

Boscolo, W.R.; Signor, A.; Feiden, A. Bombardelli, R.A., Signor, A.A.,, Reidel, A., 2005 Energia digestível para larvas de tilapia-do-nilo Oreochromis niloticus na fase de reversão sexual. Revista Brasileira de Zootecnia, 34, 1813-1818.

Brol, J., Pinho, S, M., Sgnaulin, T., Pereira, K. da R., Thomas, M.C., Mello, G.L. de, Miranda-Baeza, A., Emerenciano, M.G.C., 2017. Tecnologia de bioflocos (BFT) no desempenho zootécnico de tilápias: efeito da linhagem e densidades de estocagem. Arch. Zootec. 66, 229–235. doi:10.21071/az.v66i254.2326

Camargo, S.O., Martins, C., 2005. Parâmetros eritrocitários do jundiá (Rhamdia quelen) submetido à dieta com diferentes níveis de proteína. Ciência Rural. Rural 35, 1406–1411. doi:10.1590/S0103-84782005000600028.

Collier, H.B., 1944. Standardization of Blood Haemoglobin Determinations. Can. Med. Assoc. J. 50, 550–552.

Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., Verstraete, W., 2007. Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 270, 1–14. doi:10.1016/j.aquaculture.2007.05.006

Dabrowski, K.., Guderley, H., 2002. Intermediary metabolism., in: Fish Nutrition. Washington, pp. 309–365.

Eaton, A.D.., Cleserci, L.S., Greenberg, A.E., 1995. Standard methods for the examination of water and waste water., 10th ed. A. ed. Washington.

Ebeling, J.M., Timmons, M.B., Bisogni, J.J., 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture 257, 346–358. doi:10.1016/j.aquaculture.2006.03.019

El-Sayed, E.M., 2006. Tilapia culture., CABI publi. ed. Massachusetts, USA.

Emerenciano, M., Ballester, E.L.C., Cavalli, R.O., Wasielesky, W., 2011. Biofloc

technology application as a food source in a limited water exchange nursery system for pink shrimp Farfantepenaeus brasiliensis (Latreille, 1817). Aquac. Res. 43, 447–457. doi:10.1111/j.1365-2109.2011.02848.x

Emerenciano, M., Cuzon, G., Arévalo, M., Mascaró Miquelajauregui, M., Gaxiola, G., 2013. Effect of short-term fresh food supplementation on reproductive performance, biochemical composition, and fatty acid profile of Litopenaeus vannamei (Boone) reared under biofloc conditions. Aquac. Int. 21, 987–1007. doi:10.1007/s10499-012-9607-4

Emerenciano, M.G.C., Wasielesky, W., Soares, R.B., Ballester, E.C., Izeppi, E.M., Cavalli, R.O., 2007. Crescimento e sobrevivência do camarão-rosa (Farfantepenaeus paulensis) na fase de berçário em meio heterotrófico. Acta Sci. - Biol. Sci. 29, 1–7. doi:http://www.redalyc.org/articulo.oa?id=187115768001

Emerenciano, M.; Gaxiola, G.; Cuzon, G. Biofloc Technology (BFT): A Review for Aquaculture Application and Animal Food Industry. In: Intech. [s.l: s.n.].

Fitzsimmons, K., Martinez-Garcia, R., Gonzales-Alanis, P., 2011. Why tilapia is becoming the most important food fish on the planet., in: Proceedings of the Ninth International Symposium in Tilapia in Aquaculture. Shanghai, China., p. 2011.

Furuya, W.M., Botaro, D., Maria, R., Macedo, G. De, Gomes, V., Carolina, L., Silva, R., Silva, T.D.C., Rossetto, V., Furuya, B., Juliana, P., Sales, P., 2005. Aplicação do Conceito de Proteína Ideal para Redução dos Níveis de Proteína em Dietas para Tilápia-do-Nilo (Oreochromis niloticus). Rev. Bras. Zootec. 35, 1433–1441. doi:10.1590/S1516-35982005000500002

Furuya, W.M., Hayashi, C., Furuya, V.R.B., Soares, C.M., 2000. Exigência de proteína para alevino revertido de tilápia do Nilo (Oreochromis niloticus). Rev. Bras. Zootec. 29, 1912–1917.

Gallagher, M.L., 1999. Growth response, tissue composition, and liver enzymes changes in juvenile sunshine bass Morone chrysops × M. saxatilis, associated with dietary protein and lipid level. J. Appl. Aquac. 9, 41–51.

Goldenfarb, P.B., Bowyer, F.P., Hall, E., Brosious, E., 1971. Reproducibility in the hematology laboratory: the microhematocrit determination. Am. J. Clin. Pathol. 56, 35–39.

Gonçalves, G.S., Pezzato, L.E., Barros, M.M., Julia, M., Rosa, S., 2009. Níveis de proteína digestível e energia digestível em dietas para tilápias-do- nilo formuladas com base no conceito de proteína ideal. Rev. Bras. Zootec. 38, 2289–2298.

505 doi:10.1590/S1516-35982009001200001
506 Haridas, H., Verma, A.K., Rathore, G., Prakash, C., Banerjee, P., 2017. Enhanced
507 growth and immuno-physiological response of Genetically Improved Farmed
508 Tilapia in indoor biofloc units at different stocking densities. Aquac. Rese 1–10.
509 doi:10.1111/are.13256
510 Hu, Z., Lee, J.W., Chandran, K., Kim, S., Brotto, A.C., Khanal, S.K., 2015. Effect of
511 plant species on nitrogen recovery in aquaponics, Bioresource Technology.
512 Elsevier Ltd. doi:10.1016/j.biortech.2015.01.013
513 Ishikawa, N.M., Ranzani-Paiva, M.J.T., Lombardi, J. V., 2008. Metodologia para
514 quantificação de leuccóciotos totais em peixe Oreochromis Niloticus. Arch. Vet.
515 Sci. 13, 54–63. doi:http://hdl.handle.net/11449/70526
516 Junior, A.C.F., Carvalho, P.L.P.F. De, Pezzato, L.E., Koch, J.F.A., Teixeira, C.P.,
517 Cintra, F.T., Damasceno, F.M., Amorin, R.L., Padovani, C.R., Barros, M.M., 2016.
518 The effect of digestible protein to digestible energy ratio and choline
519 supplementation on growth, hematological parameters, liver steatosis and size-
520 sorting stress response in Nile tilapia under field condition. Aquaculture 456, 83–
521 93. doi:10.1016/j.aquaculture.2016.02.001
522 Kishida, N., Tsuneda, S., Sakakibara, Y., Kim, J.H., Sudo, R., 2008. Real-time control
523 strategy for simultaneous nitrogen and phosphorus removal using aerobic granular
524 sludge. Water Sci. Technol. 58, 445–450. doi:10.2166/wst.2008.410
525 Lima-junior, S.E., Cardone, I.B., Goitein, R., 2002. Determination of a method for
526 calculation of Allometric Condition Factor of fish. Acta Sci. 24, 397–400.
527 doi:http://hdl.handle.net/11449/67090
528 Likongwe, J.S., Stecko, T.D., Stauffer, J.R., Carline, R.F., 1996. Combined effects of
529 water temperature and salinity on growth and feed utilization of juvenile Nile
530 tilapia *Oreochromis niloticus* (Linneaus) 146, 37-46. doi:10.1016/S0044-
531 8486(96)01360-9
532 Martínez-Córdova, L.R., Emerenciano, M., Miranda-Baeza, A., Martínez-Porchas, M.,
533 2015. Microbial-based systems for aquaculture of fish and shrimp: An updated
534 review. Rev. Aquac. 7, 131–148. doi:10.1111/raq.12058
535 Martino, R.C., Cyrino, J.E.P., Portz, L., Trugo, L.C., 2002. Effect of dietary lipid level
536 on nutritional performance of the surubim, Pseudoplatystoma coruscans.
537 Aquaculture 209, 209–218. doi:10.1016/S0044-8486(01)00738-4
538 Meyer, G., Fracalossi, D.M., 2005. Estimation of jundiá (Rhamdia quelen) dietary

539 amino acid requirements based on muscle amino acid composition. Sci. Agric. 62,
540 401–405. doi:10.1590/S0103-90162005000400015
541 Morales, A.E., Cardenete, G., Abellán, E., García-Rejón, L., 2005. Stress-related
542 physiological responses to handling in common dentex (Dentex dentex Linnaeus,
543 1758). Aquac. Res. 36, 33–40. doi:10.1111/j.1365-2109.2004.01180.x
544 NRC, 1993. Nutrient requirements of fish 1993, The Nation. ed. Washington.
545 doi:10.1016/0022-0981(94)90094-9
546 Pérez-Fuentes, J.A., Hernández-Vergara, M.P., Pérez-Rostro, C.I., Fogel, I., 2016. C:N
547 ratios affect nitrogen removal and production of Nile tilapia Oreochromis niloticus
548 raised in a biofloc system under high density cultivation. Aquaculture 452, 247–
549 251. doi:10.1016/j.aquaculture.2015.11.010
550 Pezzato, L.E.; Miranda, E.C.; Barros, M.M., Pinto, L.G.Q., Furuya, W.M., Pezzatoet
551 A.C., 2002. Digestibilidade aparente de ingredientes pela tilápia do Nilo
552 (Oreochromis niloticus). Revista Brasileira de Zootecnia. 31, 1595-1604.
553 Portz, L., Cyrino, J.E.P., Martino, R.C., 2001. Growth and body composition of juvenile
554 largemouth bass Micropterus salmoides in response to dietary protein and energy
555 levels. Aquac. Nutr. 7, 247–254. doi:10.1046/j.1365-2095.2001.00182.x
556 RANZANI-PAIVA, M.T.., PÁDUA, S.B.D.., TAVARES-DIAS, M.., EGAMI, M.,
557 2013. Métodos para análise hematológica em peixes. 1ª Ed., 1ª Ed., Ed. ed.
558 Maringá.
559 Rodrigues de Lima, E.C., Liano de Souza, R., Faria de wambach, X., Lima Silva, U.,
560 De Souza Correa, E., 2015. Culture of the Nile tilapia " Oreochromis
561 niloticus " in biofloc system with different stocking densities. Rev. Bras.
562 Saúde Prod. Anim 16, 948–957. doi:10.1590/S1519-99402015000400018
563 Rosenfeld, G., 1947. Corante pancromatico para hematología e citologia humana. Nova
564 combinaçao dos componentes do May-Grünwald e do Giemsa num so corante de
565 emprego rapido. Meml. Inst. Butantan 20, 328–334.
566 Simões, M.R., Fátima, C. De, Ribeiro, A., Amaral, C., Park, K.J., Elizabeth, F., Murr,
567 X., 2007. Composição físico-química , microbiológica e rendimento do filé de
568 tilápia tailandesa (Oreochromis niloticus). Ciência e Tecnol. Aliment. 27, 608–
569 613. doi:10.1590/S0101-20612007000300028
570 Wille, K., McLean, E., Goddard, J.S., Byatt, J.C., 2002. Dietary lipid level and growth
571 hormone alter growth and body conformation of blue tilapia, Oreochromis aureus.
572 Aquaculture 209, 219–232. doi:10.1016/S0044-8486(01)00754-2

573 Wu, G., 2013. Amino acids: biochemistry and nutrition, CRC Press. ed. Boca Raton.
574 Yamamoto, T., Sugita, T., Furuita, H., 2005. Essential amino acid supplementation to
575 fish meal-based diets with low protein to energy ratios improves the protein
576 utilization in juvenile rainbow trout Oncorhynchus mykiss. Aquaculture 246, 379–
577 391. doi:10.1016/j.aquaculture.2005.02.013
578 Zhang, N., Luo, G., Tan, H., Liu, W., Hou, Z., 2016. Growth, digestive enzyme activity
579 and welfare of tilapia (Oreochromis niloticus) reared in a biofloc-based system
580 with poly-β-hydroxybutyric as a carbon source. Aquaculture 464, 710–717.
581 doi:10.1016/j.aquaculture.2016.08.013
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Tabela 1; Diet formulation and composition with different levels of digestible energy (DE) and digestible protein (DP) for Nile tilapia juveniles raised in brackish biofloc water (10 ppt) during 42 days

Treatment / Ingredients	22:3000	22:3150	22:3300	26:3000	26:3150	26:3300	30:3000	30:3150	30:3300
Brazilian waste fish meal[1]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soybean meal	33.19	33.79	34.35	44.34	45.02	45.58	55.49	56.06	56.72
Ground corn	38.99	35.75	32.56	29.65	26.32	23.12	20.29	17.08	13.79
Wheat bran	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Soy oil	2.30	4.95	7.60	1.31	3.99	6.64	0.34	2.99	5.65
L-Lysine[2]	0.87	0.86	0.84	0.45	0.43	0.42	0.03	0.02	0.00
DL-Methionine[3]	0.37	0.37	0.37	0.31	0.31	0.31	0.26	0.26	0.26
L-Threonine[4]	0.34	0.34	0.34	0.18	0.17	0.17	0.01	0.01	0.00
Premix[5]	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Vitamin C[6]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Calcareous	0.02	0.02	0.02	0.10	0.10	0.10	0.18	0.18	0.18
Dicalcium phosphate	1.50	1.50	1.50	1.24	1.24	1.24	0.98	0.98	0.98
Antioxidant[7]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Binder[8]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Calculated composition									
DP (%)	22.01	22.02	22.02	26.02	26.06	26.06	30.03	30.03	30.07
DE (kcal kg^{-1})	3001.00	3150.93	3301.04	3000.39	3152.80	3302.63	3000.91	3150.76	3302.00
Lipids (%)	5.25	7.78	10.32	4.11	6.67	9.21	2.99	5.53	8.07
Crude fiber (%)	3.86	3.83	3.81	4.29	4.27	4.24	4.72	4.69	4.67
Minerals (%)	4.55	4.54	4.53	5.08	5.08	5.07	5.61	5.60	5.60
Lysine (%)	2.09	2.09	2.09	2.09	2.09	2.09	2.09	2.09	2.09
Methionine (%)	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62
Threonine (%)	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16
Cacium (Ca) (%)	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Phosphorus (P) (%)	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83
DP:DE	13.6	14.3	15.0	11.5	12.1	12.7	10.0	10.5	11.0

Agroforte, Laguna, SC, Brazil
[2]Novus, Indaiatuba, SP, Brazil
[3]Novus, Indaiatuba, SP, Brazil
[4]Evonik, São Paulo, SP, Brazil
[5]DSM-Roche: Vitamin A, 24000 UI; D3, 6000 UI; E, 300 mg; K3, 30 mg; B1, 40 mg; B2, 40 mg; B6, 35 mg; B12, 80 mg; folic acid, 12 mg; pantothenate Ca, 100 mg; vitamin C, 600 mg; biotin, 2 mg; choline, 1.000 mg; iron, 200 mg; copper, 35 mg; manganese, 100 mg; zinc, 240 mg; iodine, 1.6 mg; cobalt, 0.8 mg; Campinas, SP, Brazil
[6]DSM-Roche, Acorvit 35, Ascorbic acid 35%, Campinas, SP, Brazil
[7]Vulkanox® BHT, 2,6 Di-tert-butyl-P-cresol, Germany
[8]Unflavored gelatin, Dr Oetker, São Paulo, SP, Brazil

Table 1; Parameters of water quality for tilapia culture evaluating different levels of protein and energy in a biofloc system with salinity of 10 ppm

Parameters	22:3000	22:3150	22:3300	26:3000	26:3150	26:3300	30:3000	30:3150	30:3300	Macrocosm
Dissolved oxygen (mg L^{-1})	6.07 ± 0.98 (3.60 - 7.60)	6.16 ± 1.21 (3.30 - 8.10)	6.11 ± 1.08 (3.40 - 7.70)	6.06 ± 1.03 (3.40 - 7.60)	6.13 ± 1.04 (3.70 - 7.70)	6.17 ± 1.08 (3.60 - 7.80)	6.00 ± 1.25 (3.40 - 7.80)	6.05 ± 1.05 (3.70 - 8.00)	6.09 ± 1.09 (3.40 - 7.90)	6.07 ± 1.07 (3.4 - 8.1)
Temperature (ºC)	26.42 ± 3.22 (22.10 - 33.20)	26.40 ± 3.25 (21.50 - 33.20)	26.29 ± 3.05 (21.70 - 33.20)	26.41 ± 3.16 (22.00 -33.30)	26.35 ± 3.16 (22.00 - 32.90)	26.30 ± 3.12 (21.20 - 33.30)	26.26 ± 2.96 (22.10 - 33.10)	26.20 ± 3.05 (21.30 ± 32.70)	26.18 ± 2.95 (21.30 - 32.80)	26.35 ± 3.22 (21.7 - 34.5)
pH	7.56 ± 0.17 (7.27 - 7.86)	7.59 ± 0.17 (7.25 - 7.86)	7.58 ± 0.17 (7.28 - 7.83)	7.58 ± 0.17 (7.30 - 7.87)	7.58 ± 0.17 (7.30 - 7.86)	7.59 ± 0.17 (7.29 - 7.85)	7.58 ± 0.17 (7.32 - 7.86)	7.59 ± 0.17 (7.33 - 7.88)	7.59 ± 0.16 (7.30 - 7.85)	7.54 ± 0.19 (7.06 - 7.87)
Ammonia (mg L^{-1})	-	-	-	-	-	-	-	-	-	0.34 ± 0.06 (0.81 - 0.01)
Nitrite (mg L^{-1})	-	-	-	-	-	-	-	-	-	0.03 ± 0.01 (0.06 - 0.01)
Nitrate (mg L^{-1})	-	-	-	-	-	-	-	-	-	0.32 ± 0.02 (0.57 - 0.03)
Orthophosphate (mg L^{-1})	-	-	-	-	-	-	-	-	-	17.34 ± 4.05 (38.1 - 6.70)
Alkalinity (mg L^{-1} of $CaCO_3$)	-	-	-	-	-	-	-	-	-	61.33 ± 3.05 (32 - 100)
Settling solids (mL L^{-1})	-	-	-	-	-	-	-	-	-	10.12 ± 9.95 (0.5 - 32)

Average ± Standard deviation, maximum and minimum

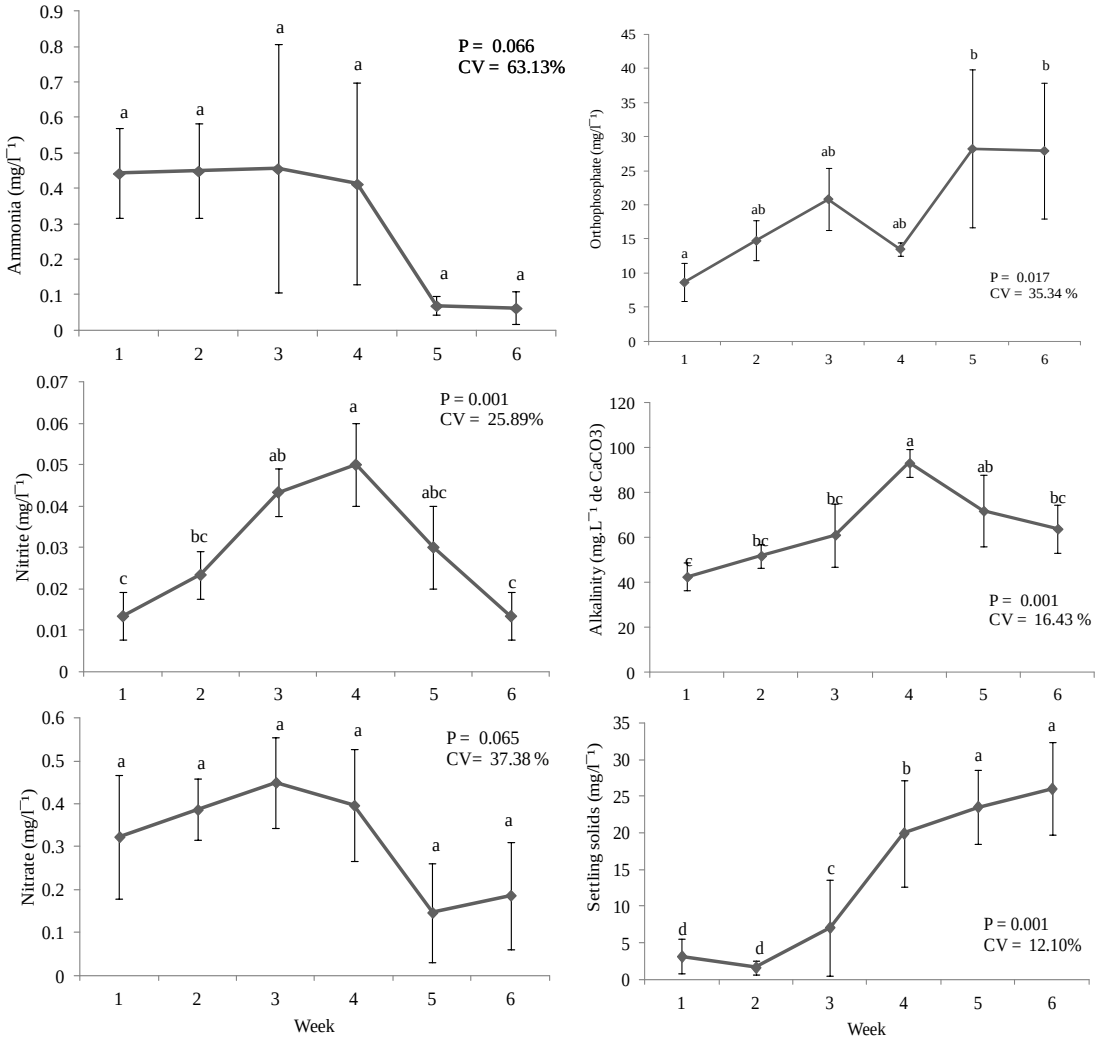

Figure 1; Variation of nitrogen compounds, alkalinity, orthophosphate and flake volume during the six experimental weeks, evaluating different levels of protein and energy for tilapia in a biofloc system with salinity of 10 ppm.

Table 2; Performance parameters of tilapia fed with different energy and protein levels in saline biofloc system 10 ppm

Protein	Energy	CP (cm)	IHS	IVS	RC (%)	FC
22		5.92 b	2.51 a	17.08	82.91	1.71 a
26		6.12 a	2.17 b	16.97	83.02	1.80 b
30		5.98 ab	2.15 b	16.33	83.67	1.80 b
	3000	6.08 A	2.17	16.21	83.79	1.79
	3150	6.07 A	2.19	16.74	83.26	1.74
	3300	5.87 B	2.47	17.44	82.56	1.78
	Protein	0.049	0.039	NS	NS	0.028
P	Energy	0.016	NS	NS	NS	NS
	Interaction	NS	NS	NS	NS	NS

Average ± Standard deviation. CP: standard length; IHS: somatic hepato index; IVS: Viscera Somatic index; RC: carcass yield; FC: condition factor. Capital letters indicate difference between protein levels and small letters indicate difference between energy levels, ($P < 0.05$) by Tukey's test.

Table 3; Performance indices of tilapia fed with different levels of protein and energy in a biofloc system with water 10 ppm of salinity.

Protein	Energy	PF (g)	CAA	SOB (%)	TCE (%/dia)	PRO (kg/m^3)	TEP
22		4.47 a	1.78	90.56	2.79 a	0.81 a	2.33 a
26		4.95 b	1.67	91.67	3.04 b	0.91 b	2.13 a
30		4.80 b	1.69	92.22	2.99 b	0.88 ab	1.79 b
	3000	4.87	1.67	92.22	3.01	0.89	2.15
	3150	4.74	1.68	92.22	2.97	0.88	2.11
	3300	4.61	1.78	90.00	2.84	0.83	2.00
	Protein	0.003	NS	NS	0.033	0.023	<0.001
P	Energy	NS	NS	NS	NS	NS	NS
	Interaction	NS	NS	NS	NS	NS	NS

Average ± Standart deviation. PF: Final weight; CAA: Apparent feed conversion; SOB: Survival; TCE: Specific growth rate; PRO: Productivity; TEP: protein efficiency ratio. Capital letters indicate difference between protein levels, (P <0.05) by Tukey's test.

Table 4; Bromatological composition of tilapia which were fed with different levels of energy and protein in a biofloc system with 10 ppm of salinity

Protein	Energy	PB	MS	MM	FAT
22		12,99 ± 0,71 A	23,29 ± 2,71	4,31 ± 0,34	5,56 ± 1,55 A
26		13,99 ± 1,01 B	22,84 ± 0,71	4,10 ± 0,20	5,25 ± 1,30 A
30		14,83 ± 0,96 C	24,01 ± 0,54	4,27 ± 0,33	4,66 ± 0,87 B
	3000	13,63 ± 0,96	22,96 ± 1,23	4,26 ± 0,33	3,80 ± 0,97 a
	3150	13,96 ± 1,35	23,31 ± 2,19	4,35 ± 0,31	5,25 ± 0,62 b
	3300	14,00 ± 1,28	23,88 ± 1,46	4,11 ± 0,21	6,08 ± 1,00 c
	Protein	<0,0001	NS	NS	0,0007
P	Energy	NS	NS	NS	<0,0001
	Interaction	0,0027	NS	NS	0,0001

Average ± Standard deviation. PB: Crude protein; MS: Dry matter; MM: Mineral matter. Capital letters indicate difference between protein levels and small letters indicate difference between energy levels. (Tukey P <0.05)

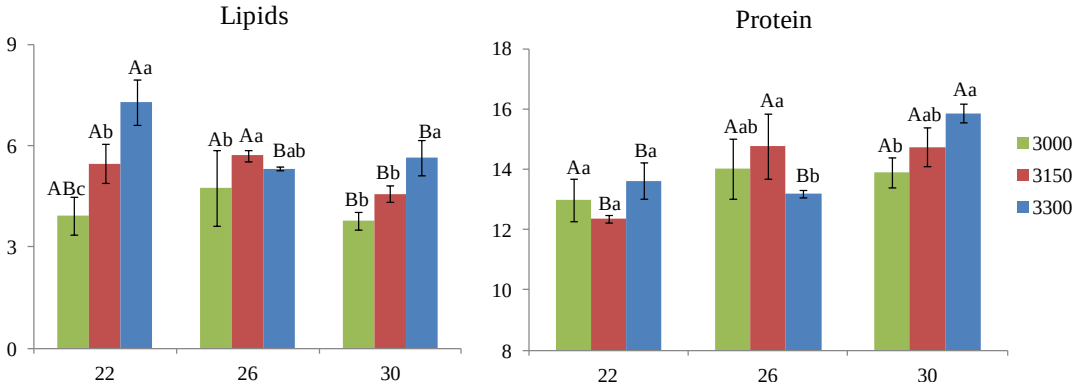

Figure 2; Lipids and protein in tilapia fingerling carcass fed with different protein and energy levels maintained in bioflocs with 10 ppm of salinity. Average ± Standard deviation. Small letters present difference between the same protein levels and different energy level. Capital letters present difference between the same energy level and different levels of protein (Tukey P <0.05).

Table 5; Hematological parameters (average ± standard deviation) of tilapia fed with different energy and protein levels in a biofloc system with 10 ppm salinity.

Protein	Energy	HEM (g/dl)	ERI	VCM	HCM	LEU (x 10^4/µL)	MON	NEU	LIN
22		7.14 ± 0.96	188.32 ± 37.68	4.45 ± 1.15	0.39 ± 0.11	7.30 ± 2.96	1.82 ± 1.30 ab	4.52 ± 3.22 b	65.32 ± 33.49
26		7.04 ± 1.25	187.44 ± 42.95	4.54 ± 1.11	0.39 ± 0.12	8.20 ± 5.91	2.53 ± 2.82 a	9.97 ± 6.25 a	62.92 ± 35.80
30		7.11 ± 1.51	193.89 ± 54.49	4.51 ± 1.66	0.38 ± 0.11	6.86 ± 2.39	0.85 ± 0.98 b	4.08 ± 3.48 b	60.64 ± 21.70
	3000	7.19 ± 0.97	203.60 ± 42.50 A	4.09 ± 0.86 A	0.36 ± 0.08	8.42 ± 5.48	2.12 ± 2.71	6.07 ± 5.54	67.58 ± 32.48
	3150	6.93 ± 1.46	176.48 ± 46.71 B	5.00 ± 1.68 B	0.41 ± 0.14	7.34 ± 3.65	1.52 ± 1.84	6.55 ± 5.88	62.00 ± 36.60
	3300	7.15 ± 1.28	189.56 ± 43.51 AB	4.42 ± 1.13 AB	0.38 ± 0.11	6.61 ± 2.31	1.57 ± 1.43	5.96 ± 4.27	59.29 ± 21.30
	Protein	NS	NS	NS	NS	NS	0.0014	<0.0001	NS
P	Energy	NS	0.039	0.011	NS	NS	NS	NS	NS
	Interaction	NS	NS	NS	NS	NS	NS	NS	NS

HEM: Hemoglobin; ERI: Erythrocyte; VCM: mean corpuscular volume; HCM: Mean corpuscular hemoglobin; LEU: Leukocytes. MON: Monocytes; NEU: Neutrophils; LIN: Lymphocytes. Small letters indicate the difference among protein levels and capital letters indicate difference among energy levels. (p, 0.05).

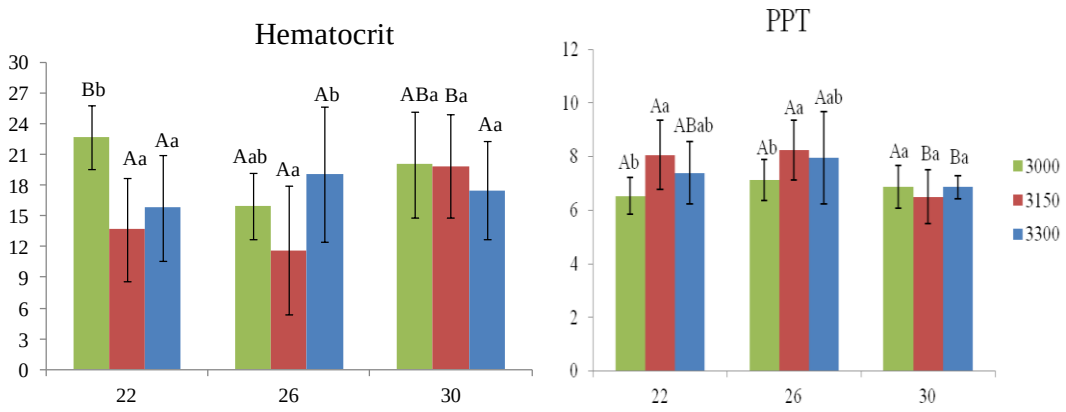

Figure 3; Blood parameters of tilapia fingerlings fed with different levels of energy and protein created in a biofloc system with 10 ppm of salinity. Average ± Standard deviation; PPT: Plasma protein; Small letters indicate difference between the same level of protein and different energy levels, capital letters indicate difference between the same level of energy and different levels of protein ($P < 0.05$)

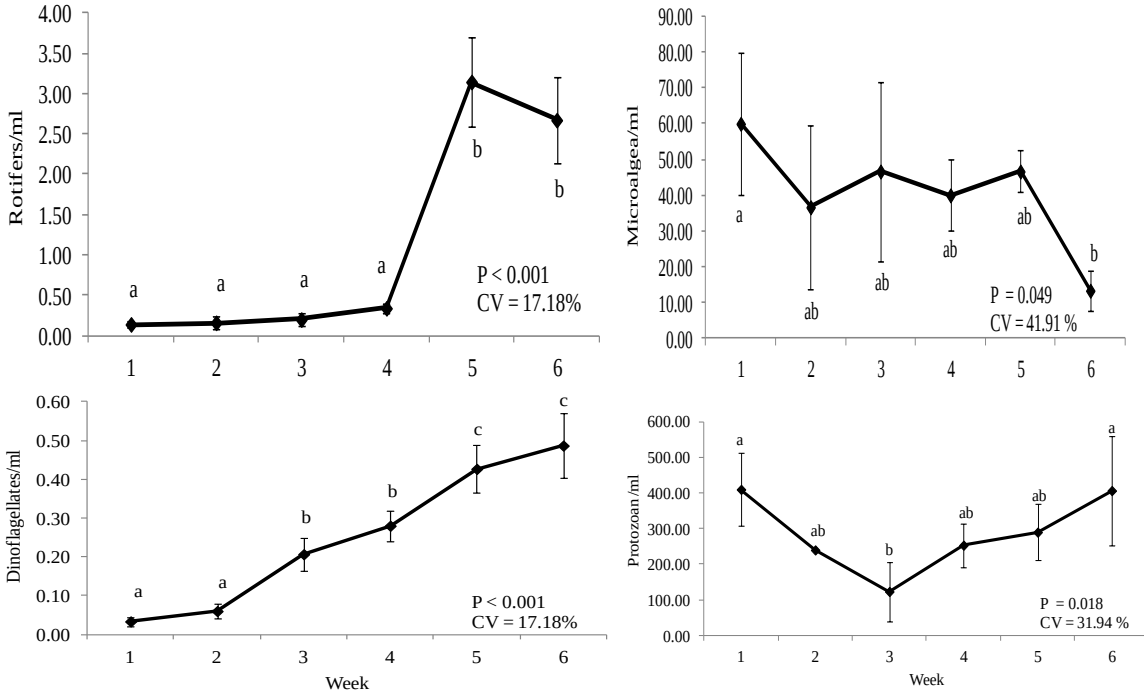

Figure 4; Abundance Averages (number of microorganisms.L-1) of water's microorganisms from tilapia culture with different levels of protein and energy in a biofloc system with brackish water. CV = Coefficient of variation, different letters in the same group of microorganisms differ statistically (P <0.05) over the 6 weeks.

2.2 – MANUSCRITO II

Enzimas digestivas e parasitologia de juvenis de tilápia-do-nilo criados em biofloco com água salobra e alimentados com diferentes níveis de energia digestível e digestível

Autores: Emerson Giuliani Durigon, Ana Paula Gottlieb Almeida, Gabriela Tomas Jerônimo, Bernardo Baldisserotto, Maurício Gustavo Coelho Emerenciano

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Research Article:

Digestive enzymes and parasitology of Nile tilapia juveniles raised in brackish biofloc water and fed with different digestible protein and digestible energy levels

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Abstract: Biofloc technology (BFT) system is increasing around the world and Nile tilapia is suggested as the major fish species for such technology. The objective of this study was to evaluate diets with different levels of digestible protein (22, 26 and 30% DP) and digestible energy (3000, 3150 and 3300 kcal kg^{-1}) for Nile tilapia juveniles raised in brackish biofloc system water (10 ppt) and its effects on the activity of digestive enzymes and the presence of ectoparasites in the gills. Tilapia juveniles (1.25 ± 0.15g of initial weight) were stocked in thirty-two experimental units (100 L tanks) with 15 fish per tank. After 42 days fish were harvested, sampled and analyzed. The pepsine (stomach) and trypsine (intestine) activities significantly increased according to the increase of dietary levels. For both cases the higher activity was observed in 30%DP treatment ($P<0.05$), with (pepsine) and without interaction (trypsine) with digestible energy levels. The opposite trend was observed for chymotripsine activity (intestine), in which 22%DP presented the higher activity, with significant interaction with energy levels. The higher activity for intestinal lipase activity was observed in 3150 kcal kg^{-1}. No difference was found for ectoparasite analyses in gills between the treatments. Although in all treatments monogea type was found (70-90% of prevalence), the number of parasites per fish was low in all treatments (less than 1.6 parasites $fish^{-1}$). The results suggested that differences in dietary digestible protein and digestible energy affects the activity of digestive enzymes in Nile tilapia juveniles raised in biofloc with 10ppt of salinity. On the other hand, biofloc system did not promote ectoparasite spread even with low dietary levels of digestible protein and digestible energy.

Keywords: BFT, nutrition, disease, monogea

1. **INTRODUCTION**

The increasing world population and the concern for the preservation of natural resources have driven to the use of "environmental friendly" intensive aquaculture systems that do not harm the environment. A clear example is the biofloc technology (BFT) system, developed initially in the 1970s for the production of marine shrimp with minimal water exchange, and from 80's to 90's also developed for fish (Emerenciano et al., 2013). Nowadays, the use of such technology has been investigated for several fish species such as *Brycon orbignyanus* (Sgnaulin et al., 2018), *Piaractus brachypomus* (Cristina et al., 2017), *Rhamdia quelen* (Poli et al., 2015), *Mugil cf. hospes* (Rocha et al., 2012), *Cyprinus carpio* (Zhao et al., 2014), and Nile tilapia *Oreochromis niloticus* (Luo et al., 2014; Long et al., 2015)

Nile tilapia is known as a species with rapid growth, rusticity, easy adaptation to intensive systems and good acceptance of commercial diets (Fitzsimmons, et al., 2011). Tilapia is well accepted by the global consumer market, as it presents a fillet without intramuscular spines, white flesh and firm texture (Simões et al., 2007). In addition, Nile tilapia is a filtering fish that can consume the microorganisms present in the bioflocs (Rodrigues et al., 2015). This factor contributes greatly to its adaptation and performance in BFT since such system has nutrient-rich live food available 24 hours per day (Martínez-Córdova et al., 2015). Another important factor is its adaptation to waters with low salinity (Souza et al., 2010), which is an alternative in different regions for polyculture with shrimps or even monoculture in shrimp farms that are not operating because spread of diseases.

In order to evaluate the production of tilapia in biofloc systems, some studies have tested stocking density (Rodrigues de Lima et al., 2015; Brol et al., 2017; Haridas et al., 2017), dietary protein levels (Azim and Little, 2008; Abdel-Tawwab et al., 2010), carbon and nitrogen ratios (Pérez-Fuentes et al., 2016) and different carbon sources (Zhang et al., 2016). However, there are no studies regarding to the digestible protein and energy levels, and its relation with digestive enzymes activity and presence of parasites in tilapias raised in biofloc systems. Some studies evaluated some specific points such as Luo et al. (2014) and Long et al. (2015), which compared the activity of digestive enzymes of tilapia raised in recirculating aquaculture (clear water) and biofloc systems.

The presence of parasites in intensive production systems is a problem that can cause

serious damage (Leira et al., 2017). On the other hand, the activity of digestive enzymes is an important parameter when the efficiency of nutrient intake need to be evaluated (Kolkovski, 2001), even more when testing feeds with different protein levels and/or plant-based protein sources (Moreno-Arias et al 2017) whether high concentration of antinutritional factors can reduce the availability of one or more nutrients and adversely affect the fish health (Mérida et al., 2010). The analysis of the digestive enzymes allows to infer about the dietary protein quality and the efficiency of the digestive system, which reflect on the fish performance (Sunde et al., 2004). In this sense, the objective of the present study was to evaluate diets with different levels of digestible protein and digestible energy for Nile tilapia (*O. niloticus*) juveniles raised in brackish biofloc system water (10 ppt) and its effects on the activity of digestive enzymes and the presence of parasites in the gills.

2. MATERIALS AND METHODS

Local and experimental design

Nile tilapia *(O. niloticus)* with 1.25 ± 0.15g initial weight were raised in the Aquaculture Laboratory at the Universidade do Estado de Santa Catarina (UDESC), city of Laguna, Santa Catarina state, Brazil. The experiment was carried out in a 3 x 3 factorial design, with isophosphoric and isocalcitics diets with 3 levels of digestible protein (22, 26, and 30% DP) and 3 levels of digestible energy (3000 3150, and 3300 kcal DE), totaling 9 treatments with 4 replicates (Table 1).

Thirty-six experimental units (100 L tanks) with a useful volume of 75 L were equally divided in three indoor stands, and 15 fish were placed per tank. After fish stocking the experimental period lasted 42 days. Each worktop was connected to a macrocosm (1000 L plastic tanks) containing a submerged pump, which pumped water to the respective stands, returning by gravity (Sgnaulin et al 2018). To maintain the quality of the biofloc, the three macrocosm tanks were connected to each other and to each stand adopting a "macrocosm-microcosms" model with continuous water circulation (Wasielesky et al., 2006; Emerenciano et al., 2007). Each 1000 L tanks (macrocosm) contained one 200 W heater to maintain the temperature.

Initially, 300 L of a biofloc mature water (inoculum) from another tilapia culture was used and a C: N ratio of 15: 1 was maintained throughout the experimental period, being sugarcane molasses the source of carbon used and the fish feed the source of N (Brol et al., 2017). In the macrocosm, tilapia juveniles were kept in a biomass of approximately 7 kg m^{-3}, which were fed with 5% of the biomass 2 times a day (8:00 a.m. and 6:00 p.m.) with a commercial feed of 32% crude protein. The period of acclimation of the fish was 7 days, and the salinity was increased gradually until reaching 10 ppt. The aeration system was based on Emerenciano et al. (2011) with constant water movement, and each experimental unit contained independent water and air intake, and the air intake through a porous stone (20 mm in diameter and 30 mm in height) fixed in the middle of the tank. The aeration in the macrocosms was done through a ring with microperforated hose (70 cm of perimeter) in the center of the tank and fixed in the bottom. This whole aeration system was by an air blower of 2hp.

1.1

Food management and preparation of diets

The ingredients were milled, sieved (300 μm), and mixed. Then, soybean oil and about 20% water were added and again mixed until complete homogenization. The mixture was pelleted through a meat grinder and the pellets were broken until they had 2 mm granulometry. After, pellets were dried for 72 h in an oven with forced air recirculation at 55 °C. The diet formulation and composition are presented in Table 1. The feedstuff values of digestible protein and digestible energy were based on values cited by Furuya (2010). The fish were fed three times a day (8:00 a.m., 1:30 p.m., and 6:00 p.m.), being 8% of the biomass in the first two weeks and after 5% of the biomass.

Water quality parameters

Dissolved oxygen, temperature (YSI-55, Yellow Springs Instruments Inc., OH, USA) and pH (YSI-10A, Yellow Springs Instruments Inc., OH, USA) were monitored daily and remained at 6.07 ± 1.07 mg/L, 26.34 ± 3.22 °C and 7.54 ± 0.19, respectively (mean ± standard deviation). Levels of total ammonia, nitrite (NO_2), nitrate (NO_3) and orthophosphate (PO_4^{3-}) were measured weekly using a photocolorimeter (AT 100P, ALFAKIT, Florianópolis, SC, Brazil), and these parameters were maintained at 0.34 ± 0.06. 0.03 ± 0.01.

0.32 ± 0.02 and 17.34 ± 4.05 mg L^{-1}, respectively. Total alkalinity was measured using commercial kit (ALFAKIT cod. 2058 and 2460, Florianópolis, SC, Brazil) and remained at 61.33 ± 3.05 mg L^{-1}. These parameters were kept within the appropriate range for the species (El-Sayed, 2006).

The biofloc volume (settling solids) was monitored daily using the 1000 mL Imhoff cone, by sedimentation of 1 L for 20 min according to methodology described by (Eaton et al., 1995). This parameter remained at 10.12 ± 9.95 mL L^{-1}.

Enzymatic activity

After the fish were euthanized according to Ethical Commission of Animal Experimentation (CONCEA-UDESC, protocol number 8917150517), the digestive tract was collected from 10 fish per treatment. The samples were immediately identified and frozen for further analysis. Samples of the stomach, anterior and posterior intestine were homogenized in an ice bath (0.05 mg wet weight: 1 mL buffer). The homogenization buffer solution was 20 mM Tris and 10 mM phosphate, pH 7.0 in 50% (v/v) glycerol. The extract was centrifuged (1500 rpm, 4 °C, 5 min) and the supernatant was used in assays as a source of enzyme.

The activities of pepsin, trypsin, chymotrypsin, and lipase were evaluated in the aforementioned tissues. Pepsin activity was performed by the specific methods of Hidalgo et al. (1999). The pepsin substrate was 1.5% casein in 0.2 M KCl (pH 1.8). Reactions were performed at 30 ^{o}C for 40 min with 15% TCA and the optical density of the supernatant was recorded at 280 nm with tyrosine as standard. The specific activity was expressed in μmol of hydrolyzed substrate min^{-1} mg of $protein^{-1}$. The activity of trypsin and chymotrypsin were carried out by the specific methods of Hummel (1959). The trypsin substrate was 1.04 mM TAME-HCl (α-p-toluenesulfonyl-L-arginine methyl ether hydrochloride) in 0.01 M $CaCl_2$ / 0.2 M Tris-HCl (pH 8.1), incubated at 25 °C and optical density monitored at 247 nm for 60 s. The chymotrypsin substrate was 1 mM BTEE (2: 3 (v/v) N-benzoyl-L-tyrosine ethyl ester), assayed in 0.1 M $CaCl_2$ / 0.1 M Tris-HCl (pH 7.8) at 30 °C. The optical density of the supernatant was monitored at 256 nm for 60 s. Activities were expressed in μmol of arginine min^{-1} mg of $protein^{-1}$ (U mg of $protein^{-1}$) and nmol tyrosine min^{-1} mg of $protein^{-1}$ (U mg of $protein^{-1}$), respectively. The lipase activity was analyzed by the method of Gawlicka et al.

(2000). The reaction was incubated with 0.4 mM p-nitrophenyl myristate in 24 nM ammonium bicarbonate (pH 7.8) with 0.5% Triton X-100 at 30 ^{o}C for 30 min. The reaction was stopped with 10 mM NaOH and read at 405 nm. The unit was defined as a substrate of μmol hydrolysate min^{-1} mg of $protein^{-1}$ (U mg $protein^{-1}$). Protein concentrations were determined in the enzyme extracts by the method of Lowry et al., (1994) with bovine albumin as the standard to establish the specific activities of the enzymes.

Parasitological analysis

At the end of trial fish were euthanized by cranial drilling for parasitological analyses. The gills filaments were removed and later fixed in 5% formalin and the number of parasites were quantified under a stereomicroscope using marked Petri Dish (Jerônimo et al., 2011). From these data, the prevalence and mean intensity of infestation were calculated according to Bush et al., (1997)

Statistical analysis

The normality (Shapiro-Wilk's Test) and homoscedasticity (Levene's Test) of the data were verified and after they were submitted to two-way analysis of variance; afterwards significant differences between the treatments were detected through the Tukey test (Sokal and Rohlf, 1969). All data were analyzed at 95% level of significance ($P<0.05$).

3. RESULTS

The activity of digestive enzymes is shown in Table 2. The results demonstrated that the increase of dietary DP increased pepsin activity in the stomach. In addition, the highest level of DP resulted in higher trypsin activity in the intestine compared to the lower DP levels. However, the highest chymotrypsin activity was observed in the diet with the lowest DP, although lowest activity was verified in fish fed 3300 kcal DE. Moreover, overall results demonstrated that dietary DE did not affect digestible enzymes activities in tilapia fed with 22% DP, but in those fed 26% DP the lowest lipase activity was observed in fish fed 3300

kcal DE. Tilapia fed 30% DP presented the lowest pepsin and chymotrypsin activities at 3000 and 3300 kcal DE, respectively.

The infestation rate and average of ectoparasites found in Nile tilapia gills is in Table 3. Only monogenetic ectoparasites *(Anacanthorus penilabiatus)* were found. In addition, all treatments presented high level of infestation with more than 60% of infected fish. However, the number of parasites in the gills of each fish was reduced and there was no significant difference between treatments.

4. DISCUSSION

The activity of digestive enzymes in fish could vary according to different environmental factors as temperature (Munilla-Morán and Saborido-Rey, 1996; Apún-Molina et al., 2009), salinity (Lee-Shing and Shu-Fen, 1989; Sánchez-Chiang et al., 1987), pH (Márquez et al., 2012); as well as according to feeding habits (Seixas Filho et al., 2000), use of different feedstuff (Zambonino-Infante and Cahu, 2007; Lin and Luo, 2011) and feed formulations with varying levels of crude protein (Hakim et al., 2006), lipid (Bazaz, Mohd and Keshavanath, 1993) and carbohydrates (Wilson, 1994). In addition, recent studies demonstrated that different culture systems affected the digestive enzyme activity of Nile tilapia. In a study carried-out by 90 days under pond and cage farming systems, Santos et al. (2016) observed that the natural food presented in ponds may have influenced trypsin and chymotrypsin activities. In the same context biofloc technology system also contributed to enzymatic variations in fish (Luo et al., 2014; Long et al., 2015), but also in penaeid shrimp (Xu and Pan, 2013; Wang et al., 2016; Moreno-Arias et al., 2017).

The present study demonstrated an increase in pepsin activity in Nile tilapia as dietary digestible protein increased. This result was expected because this enzyme is responsible for the initial hydrolysis of the proteins in the stomach (Nelson and Cox, 2013), and is directly affect by protein levels (Melo et al., 2012). After digestion of the proteins in the stomach, the food bolus goes to the intestine whether the main enzyme responsible for the degradation is trypsin (Sabapathy and Teo, 1993). In the present study trypsin also presented the highest activity in tilapia fed with the highest digestible protein level. In a 87-d study comparing 24g

O. niloticus juveniles in RAS and in an indoor biofloc system, Luo et al. (2014) observed no differences in stomach and intestinal protease activity. Although better zootecnical performance was found in BFT compared to RAS, the natural productivity of bioflocs seems not to be enough to promote changes in proteases activity.

On the other hand, in the same study the activities of lipase in the stomach and intestine showed statistical differences between the RAS and the BFT fish (Luo et al., 2014). Higher lipase activity as also found by (Long et al., 2015). Juveniles *O. niloticus* in biofloc culture using poly-β-hydroxybutyric (PHB) as a carbon source showed significantly lower intestinal lipase activity compared to those raised in biofloc culture using glucose as a carbon source (Zhang et al. 2016). No significant difference was found in the activity of intestinal lipase of *C. carpio* grown in bioflocs with different carbon sources, but all specimens raised in bioflocs showed a higher activity of this enzyme compared to those raised in clear water (Bakhshi et al., 2018). In the present study, higher lipase activity was found in treatments with higher energy content, probably affected by the higher lipid levels in the diets. These results suggest that bioflocs as a supplemental food source, use of different carbon source, as well as the composition of diets directly affects lipase activity.

Chymotrypsin is specific for some amino acids such as phenylalanine, tyrosine, and tryptophan, and in the present study the highest activity was observed in fish fed the lowest DP (22%) and the lowest activity in those fed with the highest digestible energy level (3300 kcal). This fact may have occurred to compensate the dietary deficiency in diets with lower protein and energy levels. The same trend was observed in larvae of *Dicentrarchus labrax*, which showed higher activity of this enzyme when under food restriction (Cara et al. 2007). In addition, Lazzari et al. (2010) showed that as the amount of dietary soybean meal increased the chymotrypsin activity reduced, probably due to differences in amino acids composition and presence of antinutritional factors in soybean meal. In this sense, more studies should be done to clarify the effects of bioflocs in chymotrypsin activity.

The problems related to the infestation of fish by parasites have been increasing and caused great economic losses (Leira et al., 2017). In the present study monogenoids type were found in all treatments. These parasites are the most common in freshwater fish raised in Brazil (Zanolo and Yamamura, 2006). Their adult form usually are fixed in the fish gills

and can cause injuries, making breathing difficult and being the gateway to bacterial infections (Molnar, 1994).

Although monogenoids were found in all treatments, the mean intensity of ectoparasites in gills per fish was low (Table 3), suggesting that even under nutritional challenges (e.g. low levels of digestible protein and digestible energy) fish raised in BFT might be protected and such system could control ectoparasite spread. The ectoparasites occurrence in ~1g *O. niloticus* gills and ectoderm's mucus was only 2 per fish in BFT and 15 per fish in clear-water exchange system in 4 m^2 circular lined tanks after 60 days (Emerenciano, 2009). Moreover, Avnimelech and Glasner, (2017) compared the prevalence of different ectoparasites in high water exchange and biofloc nursery systems in 80 m^3 concrete ponds. After 12 days a severe infestation of different ectoparasites was detected, and after 48 days of monitoring the potential effects of biofloc systems was clearly demonstrated by the low degree of infestation in BFT. In this sense, BFT may be an alternative to limit the prevalence of pathogenic microorganisms into the system. More studies on this system are need to identify the effects and mechanisms involved.

5. CONCLUSION

Different dietary digestible protein and digestible energy levels affect the activity of digestive enzymes in Nile tilapia juveniles raised in biofloc with 10ppt salinity. On the other hand, biofloc system did not promote ectoparasite spread even when fish were raised under low dietary levels of digestible protein and digestible energy.

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6. REFERENCES

Abdel-Tawwab, M., Ahmad, M.H., Khattab, Y.A.E., Shalaby, A.M.E., 2010. Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus (L.).* Aquaculture 298, 267–274. doi:10.1016/j.aquaculture.2009.10.027

Apún-Molina, J.P., Santamaría- Miranda, A., Luna-González, A., Martínez-Díaz, S.F., Rojas-Contreras, M., 2009. Effect of potential probiotic bacteria on growth and survival of tilapia *Oreochromis niloticus L*., cultured in the laboratory under high density and suboptimum temperature. Aquac. Res. 40, 887–894. doi:10.1111/j.1365-2109.2009.02172.x

Avnimelech, Y., Glasner, H., 2017. Effect of bioflocs on infection of tilapia fingerlings by parasites. In: Aquaculture america San Antonio, Texas, p.26.

Bakhshi, F., Najdegerami, E.H., Manaffar, R., Tukmechi, A., Farah, K.R., 2018. Use of different carbon sources for the biofloc system during the grow-out culture of common carp (*Cyprinus carpio L*.) fingerlings. Aquaculture 484, 259–267. doi:10.1016/j.aquaculture.2017.11.036

Bazaz, Mohd, M., Keshavanath, P., 1993. Effect of feeding different levels of sardine oil on growth, muscle composition and digestive enzyme activities of mahseer, Tor khudree. Aquaculture 115, 111–119. doi:10.1016/0044-8486(93)90362-3

Brol, J., Pinho, S, M., Sgnaulin, T., Pereira, K. da R., Thomas, M.C., Mello, G.L. de, Miranda-Baeza, A., Emerenciano, M.G.C., 2017. Tecnologia de bioflocos (BFT) no desempenho zootécnico de tilápias: efeito da linhagem e densidades de estocagem. Arch. Zootec. 66, 229–235. doi:10.21071/az.v66i254.2326

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., Journal, T., 1997. Parasitology Meets Ecology on Its Own Terms : Margolis et al . Revisited Published by : Allen Press on behalf of American Society of Parasitologists Stable URL : http://www.jstor.org/stable/3284227 Accessed : 01-03-2016 11 : 24 UTC Your use of the JSTOR a. J. Parasitol. 83, 575–583.

Cara, B., Moyano, F.J., Zambonino, J.L., Fauvel, C., 2007. Trypsin and chymotrypsin as indicators of nutritional status of post-weaned sea bass larvae. J. Fish Biol. 70, 1798–1808. doi:10.1111/j.1095-8649.2007.01457.x

Cristina, S., Garcés, C., Jaime, J., González, G., Acuícola, I., 2017. Biofloc effect on juvenils Cachama blanca Piaractus brachypomus growth parameters. Med. Vet. y Zootec. 12, 170–180.

Eaton, A.D.., Cleserci, L.S., Greenberg, A.E., 1995. Standard methods for the examination of water and waste water., 10th ed. A. ed. Washington.

El-Sayed, E.M., 2006. Tilapia culture., CABI publi. ed. Massachusetts, USA.

Emerenciano, M., 2009. Effect of two different diets fish meal based and organic plant based diets in *Litopenaeus Setiferus* earlier post-larvae culture under biofloc, green-water and clear-water conditions. In: World Aquaculture. Veracruz, México. p.261.

Emerenciano, M., Ballester, E.L.C., Cavalli, R.O., Wasielesky, W., 2011. Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp Farfantepenaeus brasiliensis (Latreille, 1817). Aquac. Res. 43, 447–457. doi:10.1111/j.1365-2109.2011.02848.x

Emerenciano, M., Gaxiola, G., Cuzon, G., 2013. Biofloc Technology (BFT): A Review for Aquaculture Application and Animal Food Industry, in: Intech. doi:10.5772/53902

Emerenciano, M.G.C., Wasielesky, W., Soares, R.B., Ballester, E.C., Izeppi, E.M., Cavalli, R.O., 2007. Crescimento e sobrevivência do camarão-rosa (*Farfantepenaeus paulensis*) na fase de berçário em meio heterotrófico. Acta Sci. - Biol. Sci. 29, 1–7. doi:http://www.redalyc.org/articulo.oa?id=187115768001

Fitzsimmons, K., Martinez-Garcia, R.., Gonzales-Alanis, P., 2011. Why tilapia is becoming the most important food fish on the planet., in: Proceedings of the Ninth International Symposium in Tilapia in Aquaculture. Shanghai, China., p. 2011.

Furuya, W.M., 2010. Tabelas brasileiras para a nutrição de tilápias, Toledo: GFM. doi:10.1017/CBO9781107415324.004

Gawlicka, A., Parent, B., Horn, M.H., Ross, N., Opstad, I., Torrissen, O.J., 2000. Activity of digestive enzymes in yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*): Indication of readiness for first feeding. Aquaculture 184, 303–314. doi:10.1016/S0044-8486(99)00322-1

361 Hakim, Y., Uni, Z., Hulata, G., Harpaz, S., 2006. Relationship between intestinal brush
362 border enzymatic activity and growth rate in tilapias fed diets containing 30% or 48%
363 protein. Aquaculture 257, 420–428. doi:10.1016/j.aquaculture.2006.02.034
364 Haridas, H., Verma, A.K., Rathore, G., Prakash, C., Banerjee, P., 2017. Enhanced growth
365 and immuno-physiological response of Genetically Improved Farmed Tilapia in indoor
366 biofloc units at different stocking densities. Aquac. Rese 1–10. doi:10.1111/are.13256
367 Hidalgo, M.C., Urea, E., Sanz, A., 1999. Comparative study of digestive enzymes in fish
368 with different nutritional habits. Proteolytic and amylase activities. Aquaculture 170,
369 267–283. doi:10.1016/S0044-8486(98)00413-X
370 Hummel, B., 1959. A modified spectrophotometric determination of chymotrypsin, trypsin,
371 and thrombin. Can. J. Biochem. Physiol. 37, 1393–1399. doi:10.1139/o59-157
372 Jerônimo, G., Laffitte, L., Speck, G., Martins, M., 2011. Seasonal influence on the
373 hematological parameters in cultured Nile tilapia from southern Brazil. Brazilian J. Biol.
374 71, 719–725. doi:10.1590/S1519-69842011000400017
375 Kolkovski, S., 2001. Digestive enzymes in fish larvae and juveniles—implications and
376 applications to formulated diets. Aquaculture 200, 181–201. doi:10.1016/S0044-
377 8486(01)00700-1
378 Lazzari, R., Neto, J.R., De, F., Pedron, A., Loro, V.L., Pretto, A., Gioda, C.R., 2010. Protein
379 sources and digestive enzyme activities in jundiá (*Rhamdia quelen*). Sci. Agric. 67,
380 259–266. doi:10.1590/S0103-90162010000300002
381 Lee-Shing, F., Shu-Fen, C., 1989. Effect of salinity on the activities of digestive proteases
382 from the tilapia fish, *Oreochromis niloticus* in different culture environments. Comp.
383 Biochem. Physiol. -- Part A Physiol. 93, 439–443. doi:10.1016/0300-9629(89)90063-7
384 Leira, M.H., Reghim, L.S., Ciacci, L. da S., Cunha, L.T., Botelho, H.A., Braz, M.S., Dias,
385 N.P., Cicinato, C., Melo, C.C.V., 2017. Problemas sanitários das pisciculturas
386 brasileiras. Pubvet 11, 538–544. doi:HTTP : / /DX.DOI .ORG/10.22256/PUBVE T
387 .V11N6.538-544
388 Lin, S., Luo, L., 2011. Effects of different levels of soybean meal inclusion in replacement
389 for fish meal on growth, digestive enzymes and transaminase activities in practical diets
390 for juvenile tilapia, *Oreochromis niloticus* × *O. aureus*. Anim. Feed Sci. Technol. 168,
391 80–87. doi:10.1016/j.anifeedsci.2011.03.012

392 Long, L., Yang, J., Li, Y., Guan, C., Wu, F., 2015. Effect of biofloc technology on growth,
393 digestive enzyme activity, hematology, and immune response of genetically improved
394 farmed tilapia (*Oreochromis niloticus*). Aquaculture 448, 135–141.
395 doi:10.1016/j.aquaculture.2015.05.017
396 Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with
397 the folin phenol reagent. Anal. Biochem. 28, 265–275. doi:10.1016/0304-
398 3894(92)87011-4
399 Luo, G., Gao, Q., Wang, C., Liu, W., Sun, D., Li, L., Tan, H., 2014. Growth, digestive
400 activity, welfare, and partial cost-effectiveness of genetically improved farmed tilapia
401 (*Oreochromis niloticus*) cultured in a recirculating aquaculture system and an indoor
402 biofloc system. Aquaculture 422–423, 1–7. doi:10.1016/j.aquaculture.2013.11.023
403 Márquez, A.G., Demessence, A., Platero-Prats, A.E., Heurtau, D., Horcajada, P., Serre, C.,
404 Chang, J.-S., Férey, G., Peña-O'Shea, V.A. de la, Boissière, C., Grosso, D., Sanchez, C.,
405 2012. Green microwave synthesis of MIL-100(Al, Cr, Fe) nanoparticles for thin-film
406 elaboration. Eur. J. Inorg. Chem. 100, 5165–5174. doi:10.1002/ejic.201200710
407 Martínez-Córdova, L.R., Emerenciano, M., Miranda-Baeza, A., Martínez-Porchas, M., 2015.
408 Microbial-based systems for aquaculture of fish and shrimp: An updated review. Rev.
409 Aquac. 7, 131–148. doi:10.1111/raq.12058
410 Melo, J.F.B., Seabra, a. G.L., Souza, S. a., Souza, R.C., Figueiredo, R. a C.R., 2012.
411 Substituição do farelo de milho pela farinha de manga no desempenho da tilápia-do-
412 nilo. Arq. Bras. Med. Vet. e Zootec. 64, 177–182. doi:10.1590/S0102-
413 09352012000100025
414 Mérida, S.N., Tomás-Vidal, A., Martínez-Llorens, S., Cerdá, M.J., 2010. Sunflower meal as
415 a partial substitute in juvenile sharpsnout sea bream (*Diplodus puntazzo*) diets: Amino
416 acid retention, gut and liver histology. Aquaculture 298, 275–281.
417 doi:10.1016/j.aquaculture.2009.10.025
418 Moreno-Arias, A., López-Elías, J.A., Miranda-Baeza, A., Rivas-Vega, M.E., Martínez-
419 Córdova, L.R., Ramírez-Suárez, J.C., 2017. Replacement of fishmeal by vegetable meal
420 mix in the diets of Litopenaeus vannamei reared in low-salinity biofloc system: effect
421 on digestive enzymatic activity. Aquac. Nutr. 23, 236–245. doi:10.1111/anu.12384
422 Munilla-Morán, R., Saborido-Rey, F., 1996. Digestive enzymes in marine species. I.

Proteinase activities in gut from redfish (*Sebastes mentella*), seabream (*Sparus aurata*) and turbot (*Scophthalmus maximus*). Comp. Biochem. Physiol. - B Biochem. Mol. Biol. 113, 395–402. doi:10.1016/0305-0491(95)02057-8

Nelson, D.L., Cox, M.M., 2013. Lehninger Principles of Biochemistry 6th ed., Book. doi:10.1016/j.jse.2011.03.016

Pérez-Fuentes, J.A., Hernández-Vergara, M.P., Pérez-Rostro, C.I., Fogel, I., 2016. C:N ratios affect nitrogen removal and production of Nile tilapia *Oreochromis niloticus* raised in a biofloc system under high density cultivation. Aquaculture 452, 247–251. doi:10.1016/j.aquaculture.2015.11.010

Poli, M.A., Schveitzer, R., de Oliveira Nuñer, A.P., 2015. The use of biofloc technology in a South American catfish (*Rhamdia quelen*) hatchery: Effect of suspended solids in the performance of larvae. Aquac. Eng. 66, 17–21. doi:10.1016/j.aquaeng.2015.01.004

Rocha, A.F., Da, Abreu, P.C., Wasielesky, W., Tesser, M.., 2012. Avaliação da formação de bioflocos na criação de juvenis de tainha *Mugil Cf. Hospes* sem renovação de água. Atlântica 34, 63–74. doi:10.5088/atl.2012.34.1.63

Rodrigues de Lima, E.C., Liano de Souza, R., Faria de wambach, X., Lima Silva, U., De Souza Correa, E., 2015. Cultivo da tilápia do Nilo *Oreochromis niloticus* em sistema de bioflocos com diferentes densidades de estocagem. Rev. Bras. Saúde Prod. Anim 16, 948–957. doi:10.1590/S1519-99402015000400018

Rodrigues, R.B., Meurer, F., Silva, D.M. da;, Uczay, M., Boscolo, W.R., 2015. Tecnologia de bioflocos no cultivo de tilápia do nilo (*Oreochromis niloticus*). Acta Tecnológica 10, 75–89.

Sanbapathy, U.., Teo, L.H., 1993. A quantitative study of some digestive enzymes in the rabbitfish, Siganess canaliculatus and the seabass, *Lates calcarifer*. J. Fish Biol. 42, 585–602.

Sánchez-Chiang, L., Cisternas, E., Ponce, O., 1987. Partial purification of pepsins from adult and juvenile salmon fish *Oncorhynchus keta*. Effect of NaCl on proteolytic activities. Comp. Biochem. Physiol. 87, 793–797. doi:10.1016/0305-0491(87)90390-7

Santos, J.F., Soares, K.L.S., Assis, C.R.D., Guerra, C.A.M., Lemos, D., Carvalho, L.B., Bezerra, R.S., 2016. Digestive enzyme activity in the intestine of Nile tilapia (*Oreochromis niloticus L.*) under pond and cage farming systems. Fish Physiol.

Biochem. 42, 1259–1274. doi:10.1007/s10695-016-0215-5

Seixas Filho, J.T., Oliveira, M.G.A., Donzele, J.L., Gomide, A.T.M., Menin, E., 2000. Lipase activity in the chime of three Teleostei freshwater fish. Rev. Bras. Zootec. 29, 6–14. doi:10.1590/S1516-35982000000100002

Sgnaulin, T., de Mello, G.L., Thomas, M.C., Garcia, J.R.E., de Oca, G.A.R.M., Emerenciano, M.G.C., 2018. Biofloc technology (BFT): An alternative aquaculture system for piracanjuba *Brycon orbignyanus*? Aquaculture 485, 119–123. doi:10.1016/j.aquaculture.2017.11.043

Simões, M.R., Fátima, C. De, Ribeiro, A., Amaral, C., Park, K.J., Elizabeth, F., Murr, X., 2007. Composição físico-química , microbiológica e rendimento do filé de tilápia tailandesa (*Oreochromis niloticus*). Ciência e Tecnol. Aliment. 27, 608–613. doi:10.1590/S0101-20612007000300028

Sokal, R.R., Rohlf, F.J., 1969. Biometry: the principles and practice of statistics in biological research., San Francisco: W. H. Freeman.

Souza, T.R.P., Santos, C.J.A., Santos, D.L., Queiroz, A.C. dos S., Mendes, P.P., 2010. Desempenho zootécnico da tilapia nilótica linhagem chitralada sob influência da salinidade. Rev. Bras. Eng. Pesca 5, 10–18.

Sunde, J., Eiane, S.A., Rustad, A., Jensen, H.B., Opstvedt, J., Nygård, E., Venturini, G., Rungruangsak-Torrissen, K., 2004. Effect of fish feed processing conditions on digestive protease activities, free amino acid pools, feed conversion efficiency and growth in Atlantic salmon (*Salmo salar L.*). Aquac. Nutr. 10, 261–277. doi:10.1111/j.1365-2095.2004.00300.x

Wang, C., Pan, L., Zhang, K., Xu, W., Zhao, D., Mei, L., 2016. Effects of different carbon sources addition on nutrition composition and extracellular enzymes activity of bioflocs, and digestive enzymes activity and growth performance of *Litopenaeus vannamei* in zero-exchange culture tanks. Aquac. Res. 47, 3307–3318. doi:10.1111/are.12784

Wasielesky, W., Emerenciano, M., Ballester, E., Soares, R., Cavalli, R., Abreu, P.C., 2006. Cultivo em meios com floco microbiano: um caminha a ser percorrido. Panor. da aquicultura 16, 14–23.

Wilson, R.P., 1994. Utilization of dietary carbohydrate by fish. Aquaculture 124, 67–80. doi:10.1016/0044-8486(94)90363-8

Xu, W.J., Pan, L.Q., 2013. Dietary protein level and C/N ratio manipulation in zero-exchange culture of *Litopenaeus vannamei*: Evaluation of inorganic nitrogen control, biofloc composition and shrimp performance. Aquac. Res. 1842–1851. doi:10.1111/are.12126

Zambonino Infante, J.L., Cahu, C.L., 2007. Dietary modulation of some digestive enzymes and Metabolic processes in developing marine fish: Applications to diet formulation. Aquaculture 268, 98–105. doi:10.1016/j.aquaculture.2007.04.032

Zanolo, R., Yamamura, M.H., 2006. Parasitas em tilápias-do-nilo criadas em sistema de tanques-rede Parasitas. Semin. Ciências Agrárias 27, 281–288.

Zhang, N., Luo, G., Tan, H., Liu, W., Hou, Z., 2016. Growth, digestive enzyme activity and welfare of tilapia (*Oreochromis niloticus*) reared in a biofloc-based system with poly-β-hydroxybutyric as a carbon source. Aquaculture 464, 710–717. doi:10.1016/j.aquaculture.2016.08.013

Zhao, Z., Xu, Q., Luo, L., Wang, C., Li, J., Wang, L., 2014. Effect of feed C/N ratio promoted bioflocs on water quality and production performance of bottom and filter feeder carp in minimum-water exchanged pond polyculture system. Aquaculture 434, 442–448. doi:10.1016/j.aquaculture.2014.09.006

Tabela 1; Diet formulation and composition with different levels of digestible energy (DE) and digestible protein (DP) for Nile tilapia juveniles raised in brackish biofloc water (10 ppt) during 42 days

Treatment / Ingredients	22:3000	22:3150	22:3300	26:3000	26:3150	26:3300	30:3000	30:3150	30:3300
Brazilian waste fish meal[1]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soybean meal	33.19	33.79	34.35	44.34	45.02	45.58	55.49	56.06	56.72
Ground corn	38.99	35.75	32.56	29.65	26.32	23.12	20.29	17.08	13.79
Wheat bran	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Soy oil	2.30	4.95	7.60	1.31	3.99	6.64	0.34	2.99	5.65
L-Lysine[2]	0.87	0.86	0.84	0.45	0.43	0.42	0.03	0.02	0.00
DL-Methionine[3]	0.37	0.37	0.37	0.31	0.31	0.31	0.26	0.26	0.26
L-Threonine[4]	0.34	0.34	0.34	0.18	0.17	0.17	0.01	0.01	0.00
Premix[5]	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Vitamin C[6]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Calcareous	0.02	0.02	0.02	0.10	0.10	0.10	0.18	0.18	0.18
Dicalcium phosphate	1.50	1.50	1.50	1.24	1.24	1.24	0.98	0.98	0.98
Antioxidant[7]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Binder[8]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Calculated composition									
DP (%)	22.01	22.02	22.02	26.02	26.06	26.06	30.03	30.03	30.07
DE (kcal kg^{-1})	3001.00	3150.93	3301.04	3000.39	3152.80	3302.63	3000.91	3150.76	3302.00
Lipids (%)	5.25	7.78	10.32	4.11	6.67	9.21	2.99	5.53	8.07
Crude fiber (%)	3.86	3.83	3.81	4.29	4.27	4.24	4.72	4.69	4.67
Minerals (%)	4.55	4.54	4.53	5.08	5.08	5.07	5.61	5.60	5.60
Lysine (%)	2.09	2.09	2.09	2.09	2.09	2.09	2.09	2.09	2.09
Methionine (%)	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62
Threonine (%)	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16
Cacium (Ca) (%)	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Phosphorus (P) (%)	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83
DP:DE	13.6	14.3	15.0	11.5	12.1	12.7	10.0	10.5	11.0

Agroforte, Laguna, SC, Brazil
[2]Novus, Indaiatuba, SP, Brazil
[3]Novus, Indaiatuba, SP, Brazil
[4]Evonik, São Paulo, SP, Brazil
[5]DSM-Roche: Vitamin A, 24000 UI; D3, 6000 UI; E, 300 mg; K3, 30 mg; B1, 40 mg; B2, 40 mg; B6, 35 mg; B12, 80 mg; folic acid, 12 mg; pantothenate Ca, 100 mg; vitamin C, 600 mg; biotin, 2 mg; choline, 1.000 mg; iron, 200 mg; copper, 35 mg; manganese, 100 mg; zinc, 240 mg; iodine, 1.6 mg; cobalt, 0.8 mg; Campinas, SP, Brazil
[6]DSM-Roche, Acorvit 35, Ascorbic acid 35%, Campinas, SP, Brazil
[7]Vulkanox® BHT, 2,6 Di-tert-butyl-P-cresol, Germany
[8]Unflavored gelatin, Dr Oetker, São Paulo, SP, Brazil

Tabela 2; Activity of digestive enzymes in Nile tilapia juveniles fed with different levels of digestible protein (DP) and digestible energy (DE) raised in brackish biofloc water (10 ppt) during 42 days

DP (%)	DE (kcal)	Pepsin – stomach	Trypsin – intestine	Chymotrypsin – intestine	Lipase - intestine
22	3000	1.82 ± 1.30^{Aa}	0.56 ± 0.12	613.15 ± 159.15^{Aa}	2.30 ± 0.29^{Ba}
22	3150	0.87 ± 0.39^{Ba}	0.50 ± 0.07	613.66 ± 86.92^{Aa}	2.50 ± 0.33^{Ba}
22	3300	0.92 ± 0.81^{Ba}	0.66 ± 0.16	512.08 ± 76.68^{Aa}	2.46 ± 0.21^{Aa}
26	3000	2.76 ± 0.47^{Aa}	0.56 ± 0.11	518.29 ± 83.60^{ABa}	2.81 ± 0.30^{Aa}
26	3150	1.98 ± 0.45^{Ba}	0.53 ± 0.10	483.82 ± 116.24^{Ba}	2.96 ± 0.25^{Aa}
26	3300	2.31 ± 0.89^{Ba}	0.62 ± 0.14	501.42 ± 108.40^{Aa}	1.93 ± 0.20^{Bb}
30	3000	2.79 ± 0.61^{Ab}	0.67 ± 0.14	484.63 ± 68.94^{Ba}	2.21 ± 0.12^{Ba}
30	3150	6.95 ± 3.03^{Aa}	0.70 ± 0.19	511.27 ± 110.57^{ABa}	2.35 ± 0.33^{Ba}
30	3300	6.33 ± 2.13^{Aa}	0.64 ± 0.12	326.95 ± 78.62^{Bb}	2.25 ± 0.12^{Aa}
Means for protein					
22		1.20 ± 0.98^{a}	0.57 ± 0.14^{a}	579.63 ± 120.00^{a}	2.42 ± 0.28
26		2.35 ± 0.67^{b}	0.57 ± 0.12^{a}	501.18 ± 101.06^{b}	2.56 ± 0.52
30		5.35 ± 2.80^{c}	0.67 ± 0.15^{b}	440.95 ± 118.46^{b}	2.27 ± 0.21
Means for energy					
3000		2.45 ± 0.96	0.60 ± 0.13	538.69 ± 120.07^{a}	2.44 ± 0.36^{ab}
3150		3.27 ± 3.19	0.57 ± 0.15	536.25 ± 116.45^{a}	2.60 ± 0.39^{a}
3300		3.18 ± 2.59	0.64 ± 0.12	446.82 ± 176.32^{b}	2.22 ± 0.28^{b}
P	Protein	<0.0001	0.0043	<0.0001	NS
	Energy	NS	NS	0.0007	<0.0001
	Interaction	<0.0001	NS	0.0427	<0.0001

Mean ± standard deviation. Upper portion: different lowercase letters indicate significant difference between different digestible energy levels at the same digestible protein level. Different capital letters indicate significant difference between different levels of digestible protein at the same digestible energy level (two-way ANOVA and Tukey 'test, $P < 0.05$). Bottom: different letters indicate significant difference between treatments (two-way ANOVA and Tukey 'test, $P < 0.05$)

Tabela 3; Prevalence and mean intensity ± standard deviation of ectoparasites in Nile tilapia gills raised in brackish biofloc water (10 ppt) and fed with different levels of digestible protein (DP) and digestible energy (DE) during 42 days

DP (%)	DE (kcal)	Prevalence (%)	Mean Intensity
	3000	70	1.86 ± 0.69
22	3150	70	1.71 ± 0.76
	3300	80	2.00 ± 0.93
	3000	60	1.83 ± 0.98
26	3150	70	1.86 ± 0.90
	3300	80	1.88 ± 0.83
	3000	80	1.88 ± 0.97
30	3150	90	1.67 ± 0.71
	3300	70	1.43 ± 0.79

3. CONSIDERAÇOES FINAIS

Foi observado uma relação predador presa no sistema de biofloco, onde comforme aumentou o numero de rotíferos diminuiu o numero de micoalgas, a diminuição das micooalgas pode estar associada também há uma maior turbidez da água. Conforme ocorre a maturação do sistema a relação entre os microrganismos vai se estabelendo. Os rotíferos foram os micoorganismos que apareceram em maior abundancia ao final do experimento o que é muito importante do ponto de vista nutricional, visto que estes micoorganismos contribuen diretamente para a alimentação das tilápias

Para as variáveis de desempenho zootécnico e sanguíneas os níveis de 26% e 30% de PD foram semelhantes, isso pode ser devido ao suporte nutricional proveniente dos micoorganismos oriundos do sistema de vioflocos, pois como visto em outros trabalhos em sistema de resirculação os níveis de proteína para esta fase são mais altos do que o relatado neste trabalho. As variáveis de composição da carcaça foram influenciadas pelos níveis de proteína e energia da dieta, o que já era esperado pois conforme o aumento de proteína na dieta houve um aumento de proteína na carcaça e comforme o aumento de energia (aumento de lipídeos na dieta) houve um aumento de lipídeos na carcaça.

Os nívei de energia e proteína não influenciaram a concentração de ectoparasitas, o que mostra que o sistema de bioflocos não promove a disseminação destes mesmo quando os peixes são criados sob baixos níveis de proteína digestível e energia digestível, já os níveis de proteína digestível e energia digestível na dieta afetam a atividade de enzimas digestiva. Recomenda-se a utilização de 26% de PD e 3150 kcal ED para juvenis de tilápia do Nilo criados em bioflocos com 10ppt de salinidade

4. REFERÊNCIAS INTRODUÇÃO

ACEB. **1º Anuário Brasileiro de pesca e aquicultura**. Brasília, DF: [s.n.].

AVNIMELECH, Y.; KOCHBA, M. Evaluation of nitrogen uptake and excretion by tilapia in bio floc tanks, using 15N tracing. **Aquaculture**, v. 287, n. 1–2, p. 163–168, 2009.

AYROZA, L. M. DA S. et al. Custos e rentabilidade da produção de juvenis de tilápia-do-nilo em tanques-rede utilizando-se diferentes densidades de estocagem. **Revista Brasileira de Zootecnia**, v. 40, n. 2, p. 231–239, 2011.

AZIM, M. E.; LITTLE, D. C. The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (Oreochromis niloticus). **Aquaculture**, v. 283, n. 1–4, p. 29–35, 2008.

BAPTISTA, C. et al. **Anuário peixe BR da piscicultura 2018**. [s.l: s.n.].

BICUDO, A. J. A.; SADO, R. Y.; CYRINO, J. E. P. Growth performance and body composition of pacu Piaractus mesopotamicus (Holmberg 1887) in response to dietary protein and energy levels. **Aquaculture Nutrition**, v. 16, n. 2, p. 213–222, 2010.

BROL, J. et al. Tecnologia de bioflocos (BFT) no desempenho zootécnico de tilápias: efeito da linhagem e densidades de estocagem. **Archivos de Zootecnia**, v. 66, n. 254, p. 229–235, 2017.

CRAB, R. et al. Nitrogen removal techniques in aquaculture for a sustainable production. **Aquaculture**, v. 270, n. 1–4, p. 1–14, 2007.

CRAB, R. et al. Biofloc technology in aquaculture: Beneficial effects and future challenges. **Aquaculture**, v. 356–357, p. 351–356, 2012.

EKASARI, J. et al. Biofloc technology positively affects Nile tilapia (Oreochromis niloticus) larvae performance. **Aquaculture**, v. 441, p. 72–77, 2015.

EMERENCIANO, M. et al. Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp Farfantepenaeus brasiliensis (Latreille, 1817). **Aquaculture Research**, v. 43, n. 3, p. 447–457, 2011.

EMERENCIANO, M. G. C. et al. Biofloc Technology (BFT): A Tool for Water Quality Management in Aquaculture. In: **Intech**. [s.l: s.n.]. p. 92–109.

EMERENCIANO, M.; GAXIOLA, G.; CUZON, G. Biofloc Technology (BFT): A Review for Aquaculture Application and Animal Food Industry. In: **Intech**. [s.l: s.n.].

FAO. **El estado mundial de la pesca y la acuiculture**. [s.l: s.n.].

FERRI;, G. H. et al. Desempenho de alevinos de tilápia do nilo alimentados com diferentes níveis de proteína em sistema bioflocos. **10º Congresso Interinstitucional de Iniciação Científica**, p. 1–8, 2016.

FITZSIMMONS, K. M.; ALANIS, PABLO GONZALES. **Why tilapia is becoming the most important food fish on the planet**. [s.l: s.n.].

FURUYA, W. M. et al. Níveis de levedura desidratada “spray-dried” na dietade alevinos revertidos de tilápia do nilo *(oreochromis niloticus)*. **Ciência Rural**, v. 30, n. 4, p. 699–704, 2000.

FURUYA, W. M. et al. Aplicação do Conceito de Proteína Ideal para Redução dos Níveis de Proteína em Dietas para Tilápia-do-Nilo (Oreochromis niloticus). **Revista Brasileira de Zootecnia**, v. 35, n. 5, p. 1433–1441, 2005.

Furuya WM Tabelas Brasileiras para a Nutriçãode Tila´pias. GFM, Toledo, 2010.

HU, Z. et al. **Effect of plant species on nitrogen recovery in aquaponics**. [s.l.] Elsevier Ltd, 2015. v. 188

IBGE. **Produção da Pecária Municipal**. [s.l: s.n.].

KUBITZA, F. **Tilápia: Tecnologia e planejamento na produção comercial**. 2 ed ed. Jundiaí: [s.n.].

LEONARDO, A. F. G. et al. Avaliação econômica da produção de juvenis de tilápia-do-nilo, alimentados com ração comercial e com a produção primária advinda da adubação orgânica e inorgânica. **Custos e @gronegócio on line**, v. 5, n. 1808–2882, p. 22–35, 2009.

MEURER, F.; HAYASHI, C.; BOSCOLO, W. R. Apparent Digestibility of Some Protein Sources for Nile Tilapia (Oreochromis niloticus). **Revista Brasileira de Zootecnia**, v. 32, n. 6, p. 1801–1809, 2003.

MPA. **Boletim estatístico da pesca e aquicultura.**Brasilia, 2013.

RAY, A. J. et al. Suspended solids removal to improve shrimp (Litopenaeus vannamei) production and an evaluation of a plant-based feed in minimal-exchange, superintensive culture systems. **Aquaculture**, v. 299, n. 1–4, p. 89–98, 2010.

RIGHETTI, J. S. et al. Redução da proteína em dietas para tilápias-do-nilo por meio da suplementação de aminoácidos com base no conceito de proteína ideal. **Revista Brasileira de Zootecnia**, v. 40, n. 3, p. 469–476, 2011.

RODRIGUES DE LIMA, E. C. et al. Culture of the Nile tilapia " Oreochromis niloticus " in biofloc system with different stocking densities. **Rev. Bras. Saúde Prod. Anim**, v. 16, n. 4, p. 948–957, 2015.

SIGNOR, A. et al. Parâmetros Hematológicos Da Tilápia-Do-Nilo: Efeito Da Dieta Suplementada Com Levedura E Zinco E Do Estímulo Pelo Frio. **Ciência Animal Brasileira**, v. 11, n. 3, p. 509–519, 2010.

SIMÕES, M. R. et al. Composição físico-química , microbiológica e rendimento do filé de tilápia tailandesa (Oreochromis niloticus). **Ciência e Tecnologia de Alimentos**, v. 27, n. 3, p. 608–613, 2007.

WASIELESKY, W. et al. Cultivo de camarões em sistema de biofl ocos : realidades e perspectivas. **revista ABCC**, n. May, p. 30–36, 2013.

WHO. **Protein and amino acid requirements in human nutrition.** [s.l: s.n.].

5. CARTA DE APROVAÇÃO DO CETEA

UDESC UNIVERSIDADE DO ESTADO DE SANTA CATARINA | LAGES CENTRO DE CIÊNCIAS AGROVETERINÁRIAS

Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "Níveis proteicos e relação energia:proteína em berçário de tilápias cultivados com 10 ppm de salinidade.", protocolada sob o CEUA nº 8917150517 (ID 000333), sob a responsabilidade de **Maurício Gustavo Coelho Emerenciano** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade do Estado de Santa Catarina (CEUA/UDESC) na reunião de 26/05/2017.

We certify that the proposal "Protein levels and energy ratio: protein in tilapia nursery grown with 10 ppm of salinity.", utilizing 480 Fishes (480 males), protocol number CEUA 8917150517 (ID 000333), under the responsibility of **Maurício Gustavo Coelho Emerenciano** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the University of Santa Catarina State (CEUA/UDESC) in the meeting of 05/26/2017.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 10/2017 a 12/2017 Área: Engenharia de Pesca

Origem:	Animais provenientes de estabelecimentos comerciais				
Espécie:	Peixes	sexo: Machos	Idade:	10 a 60 dias	N: 480
Linhagem:	GIFT		Peso:	0 a 4 g	

Local do experimento: O experimento será realizado no Laboratório de Aquicultura (LAQ), da Universidade do Estado de Santa Catarina (UDESC), campus do Centro de Educação Superior da Região Sul (CERES), em Laguna, Santa Catarina, Brasil.

Lages, 27 de maio de 2018

Marcia Regina Pfuetzenreiter
Coordenadora da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

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