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Tilapia nursery stocking densities in a chemoautotrophic biofloc system

Densidade de estocagem no berçário de tilápia em sistema de bioflocos quimioautotrófico

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ABSTRACT

The nursery phase in tilapia using biofloc technology is important as it increases the predictability of production. However, none studies evaluating the stocking densities of tilapia focused only on the use of an inorganic carbon source to promote the nitrification process as the main way to control nitrogen in the system. This study aimed to evaluate the effect of varied nursery stocking densities, in a chemoautotrophic biofloc system, on water quality, zootechnical parameters, and health of Nile tilapia (*Oreochromis niloticus*). Fifteen tanks (100 L capacity) containing heaters (28 ±1°C) inoculated with mature bioflocs were used. Seven hundred and fifty tilapia fingerlings (weighing 0.66 ±0.17 g) were distributed in the tanks, in triplicate, so that the densities in the tanks reached 200, 350, 500, 650, and 800 fish m^{-3}. Dissolved oxygen and tilapia growth showed a negative linear relationship with fish density. A positive linear relationship with density was observed for nitrogen compounds, alkalinity, suspended solids, yield, and feed conversion. However, the water quality parameters were appropriate for tilapia and allowed good zootechnical performance irrespective of the fish density. Hematological parameters, fish survival, and uniformity in growth did not alter with densities. Total suspended solids produced by fish biomass showed a quadratic relationship with density, with the highest efficiency of the tanks stocked with 406 fish m^{-3}. It is possible to construct a tilapia nursery in chemoautotrophic biofloc systems with densities reaching up to 800 fish m^{-3} and yield exceeding 12 kg m^{-3}. But the density of 406 fish m^{-3} had a better efficiency in solid production by biomass.

Index terms: *Oreochromis niloticus*; juvenile production; superintensive system; yield; hematology.

RESUMO

A fase de berçário no cultivo da tilápia utilizando a tecnologia de bioflocos é importante, pois aumenta a previsibilidade da produção. No entanto, nenhum estudo avaliando as densidades de estocagem de tilápias focou no uso apenas de uma fonte de carbono inorgânico para promover o processo de nitrificação como principal forma de controle de nitrogênio no sistema. Este estudo teve como objetivo avaliar o efeito de densidades de estocagem no berçário, em sistema de bioflocos quimioautotróficos, na qualidade da água, parâmetros zootécnicos e sanidade da tilápia-do-nilo (*Oreochromis niloticus*). Quinze tanques (capacidade de 100 L) contendo aquecedores (28 ±1°C) e inoculados com bioflocos maduros foram utilizados. Setecentos e cinquenta alevinos de tilápia (pesando 0,66 ±0,17 g) foram distribuídos nos tanques, em triplicata, de forma que as densidades nos tanques atingissem 200, 350, 500, 650 e 800 peixes m^{-3}. O oxigênio dissolvido e o crescimento da tilápia mostraram uma relação linear negativa com a densidade de peixes. Uma relação linear positiva com a densidade foi observada para os compostos nitrogenados, alcalinidade, sólidos suspensos, produtividade e conversão alimentar. No entanto, os parâmetros de qualidade da água foram adequados para a tilápia e permitiram um bom desempenho zootécnico independente da densidade de estocagem. Parâmetros hematológicos, sobrevivência dos peixes e uniformidade no crescimento não se alteraram com as densidades. Os sólidos totais em suspensão produzidos pela biomassa de peixes apresentaram relação quadrática com a densidade, com maior eficiência dos tanques estocados com 406 peixes m^{-3}. É possível produzir no berçário de tilápias utilizando sistemas de bioflocos quimioautotróficos com densidades de até 800 peixes m^{-3} e produtividade superior a 12 kg m^{-3}. Mas a densidade de 406 peixes m^{-3} apresentou melhor eficiência na produção de sólidos por biomassa.

Termos para indexação: *Oreochromis niloticus*; produção de juvenil; sistema superintensivo; produtividade; hematologia.

INTRODUCTION

The nursery phase in tilapia (fishes weighing between 1–30 g) is conducted in ponds or net cages (Valenti et al., 2021). This phase is important as it increases the predictability of production (Brol et al., 2017; Silva; Massago; Marchiori, 2019) and can be carried out during the winter season to achieve weight gain in fish at the beginning of the season; improving the production scale. (Silva; Massago; Marchiori, 2019).

The biofloc technology (BFT) tackles both environmental and economic issues by protecting water resources and achieving high yields (Avnimelech, 2012). Intensive tilapia culture is considered a sustainable alternative as it allows the production of significantly higher biomass compared to the conventional flow-through systems, consuming less water, as well as limiting the release of effluents to the surroundings (Jatobá; Borges; Silva, 2019). Furthermore, the BFT is a better biosafe system as it prevents *Streptococcus* (Amal; Zamri-Saad, 2011; Chideroli et al., 2017) and *Francisella* infections (Leal; Tavares; Figueiredo, 2014; Nguyen et al., 2016) that are frequently reported from the subtropical regions of Brazil (Jatobá; Klipp; Hoppe, 2016). More recently, viruses such as Tilapia Lake Virus (TiLV) and Infectious Spleen and Kidney Necrosis Virus (ISKNV) had also caused infections in tilapia, posing a threat to the tilapia-rearing realm (Jansen; Dong; Mohan, 2018; Figueiredo et al., 2020).

Several studies have evaluated the stocking densities at various stages of tilapia and the use of different carbon sources in the biofloc system with variable results (Lima et al., 2015; Brol et al., 2017; Haridas et al., 2017; Lima et al., 2018; Liu et al., 2018; Vieira et al., 2019; Eid et al., 2020; Vicente et al., 2020; Zaki et al., 2020; Manduca et al., 2021). However, none of these studies focused on the nursery rearing phase (1–30 g), using only an inorganic carbon source to involve the nitrification process as the main way to control nitrogen in the system.

The use of the nitrification process in BFT makes the system predominantly chemoautotrophic, which provides a more stable rearing system with less solid production (Ferreira et al., 2020; Ferreira et al., 2021). In the chemoautotrophic system, the conversion of one gram of ammonia through the nitrification process can produce up to 40 times less bacterial biomass compared to the heterotrophic process (that uses organic carbon sources) with similar dissolved oxygen (DO) consumption (Ebeling; Timmons; Bisogni, 2006), facilitating system maintenance and reducing the need to remove solids. Therefore, this study aimed to evaluate the effect of varied stocking densities, during the nursery phase, in a chemoautotrophic biofloc system and monitor the water quality, zootechnical performance, and hematological parameters of juvenile Nile tilapia (*O. niloticus*).

MATERIAL AND METHODS

This study was conducted at a fish farming unit of the Epagri, located in the state of Santa Catarina, Brazil. Male Nile tilapia fingerlings (*O. niloticus*), GIFT strain, with an initial weight of 0.66 ±0.17 g, were used. All procedures were carried out following the rules and standards of the Ethics Committee on the Use of Animals and approved by CEUA n° 305/2019.

Experimental design

Rectangular experimental units (78 × 56 cm) of 100 L capacity each, equipped with central aeration (aerotube™) and individual heaters (200 W) with thermostats (28 ±1 °C), were stocked with male GIFT Nile tilapia (0.66 ±0.17 g), in triplicate, at the densities of 200 (D200), 350 (D350), 500 (D500), 650 (D650), and 800 (D800) fish m^{-3}. Initially, the experimental units were inoculated with 20% of previously matured bioflocs to obtain a final value of about 100 mg L^{-1} of solids.

The matrix tank used to inoculate the bioflocs in the experimental units had the following water parameters on the day of the transfer: temperature 30 °C, 6.35 mg L^{-1} DO, pH 7.54, 0.17 mg N-$NH_{3,4}$ L^{-1}, 0.15 mg N-NO_2 L^{-1}, 239 mg N-NO_3 L^{-1}, 78 mg L^{-1} alkalinity, 153 mg L^{-1} hardness, 28 mL of suspended solids (SS), and 498 mg L^{-1} of total suspended solids (TSS). The matrix tank (size-1 m^3) had a total of 10.5 kg of tilapia juveniles (average weight, 28 g) on the day of the transfer.

The feed, bought from the Guabi Nutrição e Saúde Animal (São Paulo, Brazil), were administered four times a day (8 am, 11 am, 2 pm, and 5 pm), adjusted according to the weekly weighing, sampling 70% of fish/tank (Table 1).

Table 1: Feed management in the nursery tank of Nile tilapia under the biofloc system.

Size of fish	Diet	%FW
0.5 a 5 g	1.0 mm, 45%CP	12.70%
5 a 10 g	1.7 mm, 36%CP	6.8%
10 a 20 g	1.7 mm, 36%CP	5.95%

Abbreviations: FW – Fish weight. CP – Crude protein.

Water quality management

The dissolved oxygen (DO), temperature, and pH were measured daily (YSI, model Pro Plus) 30 min after the first feeding. The analysis of total ammonia nitrogen (TAN), nitrite, and alkalinity was performed twice weekly. Nitrate, hardness, and salinity were estimated at the beginning (1st day), middle (24th day), and at the end of the experiment (42nd day). Salinity was measured using a Thermo Scientific meter (model Orion Star A222).

TAN, nitrite, and nitrate levels were estimated by a micro-processed photocolorimeter and a colorimetric kit (Alfakit®). The volume of ammonia was determined using the indophenol colorimetric method (4500-NH_3 F, American Public Health Association - APHA, 1995), nitrite by diazonium colorimetric method (4500-NO_2-B, APHA, 1995), and nitrate by the brucine method (Fries et al., 1977). Alkalinity and hardness of water were tested by the titration method using a colorimeter kit (Alfakit®) as per the methodology described in the APHA (1995).

During the experiment, the water loss due to evaporation from the BFT was replenished weekly. Twice a day (8 am and 5 pm), sodium bicarbonate ($NaHCO_3$) was added to the water in the experimental units to maintain its alkalinity within 60 – 80 mg L^{-1} and the pH between 7.0 – 7.5. The amount of $NaHCO_3$ added to the water was calculated based on the percentage of feed offered daily (w/w) in proportions ranging from 12% to 18% of the daily feed intake. As per the pH and alkalinity values, this proportion was adjusted accordingly.

For each experimental unit, the consumption of $NaHCO_3$ and feed intake was calculated using the following Equations 1 and 2:

$$Relative\ alkalinity\ consumption(\%) = \\ = 100 \times \left(\frac{Alkalinizing\ consumption(g)}{Feed\ intake(g)} \right) \quad (1)$$

$$Amount\ of\ base\ by\ biomass\left(g.kg^{-1}\right) = \\ = \frac{Alkalizing\ Consumption(g)}{(FB - IB)} \quad (2)$$

where FB is the final biomass (kg $tank^{-1}$), and IB is the initial biomass (kg $tank^{-1}$).

Solids management

Sedimentable solids (SS) and total suspended solids (TSS) were estimated biweekly from all experimental units using the Imhoff cone and APHA (1995) methodologies, respectively. When the TSS exceeded 600 mg L^{-1} (Schveitzer et al., 2013), solids were removed by passing water through bag filters (50 µm). The volume to be filtered was calculated by using the following Equation 3:

$$Vf = Vt - \left(\frac{TSSd \times Vt}{TSSa} \right) \quad (3)$$

where Vf is the volume to be filtered (L), Vt is the tank volume (100 L), TSSd is the desired total solids (600 mg L^{-1}), and TSSa is the total analyzed solid (mg L^{-1}).

At the end of the experiment, the total solids produced by each experimental unit and the total solids produced per kg of fish were calculated using the following Equations 4 and 5:

$$Solids\ produced\left(g.tank^{-1}\right) = (TSSf \times Vt) - \\ -(TSSi \times Vt) + \sum_{i=0}^{n} (TSSr \times Vf)\, n \quad (4)$$

$$Solids\ produced\ by\ biomass\left(g.kg^{-1}\right) = \\ = \frac{Solids\ produced\left(g.tank^{-1}\right)}{(FB - FI)} \quad (5)$$

where TSSf is the final total solids (g L^{-1}), Vt is the tank volume (100 L), TSSi is the initial total solids (g L^{-1}), TSSr is the total solids on the day of filtration (g L^{-1}), Vf is the filtered volume (L), FB is the final biomass (kg $tank^{-1}$), and IB is the initial biomass (kg $tank^{-1}$).

Zootechnical performance

On the 44th day of rearing, all experimental units were harvested to obtain data based on the following parameters: final weight, specific growth rate (SGR), daily weight gain (DWG), feed conversion ratio (FCR), total yield, survival, and uniformity. The following formulas were used for the assessments (Equations 6, 7, 8, 9, 10 and 11):

$$SGR(\%) = 100 \times \left(\frac{ln(FW) - ln(IW)}{t} \right) \quad (6)$$

$$DWG\left(g\ day^{-1}\right) = \frac{(FW - IW)}{t} \quad (7)$$

$$FCR = \frac{FI}{(FB - IB)} \quad (8)$$

$$Yield\left(\frac{kg}{m^3}\right) = \frac{FB(kg)}{V\left(m^3\right)} \quad (9)$$

$$Survival(\%) = 100 \times \frac{Nf}{Ns} \quad (10)$$

$$Uniformity(\%) = 100 \times \frac{N \pm 20\%}{Nf} \quad (11)$$

where FW is the final weight (g), IW is the initial weight (g), t is the rearing time (days), FI is feed intake (g), FB is final biomass (g), IB is initial biomass (g), V is the experimental unit volume (m^3), Nf is the total number of fish harvested, Ns is the initial number of fish stocked, and N ±20% is the number of fish with an average weight (±20%)in the experimental units (Piedras et al., 2005).

Hematological analysis

After 44 days of rearing and a 24 h period of starvation, four fish from each tank were anesthetized with Eugenol (50 mg L^{-1}), and blood was collected from the caudal vessel using a 3-mL syringe containing a drop of 10% EDTA as an anticoagulant.

For hematological analyses, hematocrit percentage was measured by the microhematocrit method, and red blood cell count (RBC) was conducted in a Neubauer chamber after diluting blood with Dacie's fluid. Blood smear slides were stained with Giemsa and May-Grünwald stains (Rosenfeld, 1947); total and differential leukocyte counts were carried out as described by Jatobá et al. (2011).

Data analysis

Data normality and homoscedasticity were analyzed using Bartlett's and Shapiro-Wilk tests, respectively. Subsequently, the effects of varied stocking densities on water quality, growth, and hematological parameters were evaluated using regression models. The adjustment of the data to the model was verified based on the significance ($p < 0.05$) of the regression coefficients by t-test, the determination coefficient (R^2 = SQReg/SQTreatment), the sum of squared deviations, and the values obtained from the study. ANOVA was also performed to verify the significance ($p<0.05$) of the regression models.

RESULTS AND DISCUSSION

Water and solid analyses

The temperature, alkalinity, and initial salinity were not altered with different stocking densities (Table 2). Water temperature and alkalinity did not vary significantly throughout the rearing process. The alkalinity values remained within an average scale (close to 70 mg L^{-1}) that is considered adequate for tilapia rearing. (Cavalcante et al., 2009).

The average initial salinity in the experimental units was 0.74 ppt. An amount of 30 g of common salt (0.3 ppt) was added to all the experimental units to prevent toxicity due to nitrite peaks during rearing (Alvarenga et al., 2018). At the end of the experiment, the salinity values showed a positive linear correlation with the stocking density, reaching an average of 2.21 ppt with the highest stock density. This is mainly due to the accumulation of sodium from the alkalinizer used ($NaHCO_3$), which was calculated and added apropos to the feed supply and the biomass of the nursery.

DO present a negative linear relationship with stocking density in the tilapia nursery (Table 2). However, the values remained above 5 mg L^{-1}, except when the biomass reached 8 kg m^{-3} and daily feed supply exceeded 500 g m^{-3} during the D650 and D800 treatments. These values were still acceptable for the rearing of tilapia juveniles (Silva; Massago; Marchiori, 2019).

Nitrogen compounds (total ammonia, nitrite, and nitrate) showed a positive linear relationship with the tilapia stocking density (Table 2). A higher feed input in the treatments correlated with the higher stocking densities. However, TAN, nitrite, and nitrate mostly remained within the acceptable limits for the growth and survival of the Nile tilapia (Monsees et al., 2017; Silva; Massago; Marchiori, 2019). The toxic ammonia (NH_3) did not show any significant variations between the experimental units throughout the experiment, and its volume remained below 0.1 mg L^{-1}. Interestingly, the amount of nitrite varied over time, especially after day 20 (Figure 1). However, D800 showed mean nitrite values above 1 mg $N\text{-}NO_2$ L^{-1} when fish biomass exceeded 8 kg m^{-3} and the daily amount of feed administered was 500 g m^{-3}; thus, suggesting a daily nitrogen intake of 28.8 g m^{-3}. The less toxic nitrate remained within satisfactory limits (Table 2). These low variations in the concentration of nitrogenous compounds throughout the experiment are indications of mature BFT in the chemotrophic stage (Ebeling et al., 2006).

While experimenting with different stocking densities of the Nile tilapia and BFT, Haridas et al. (2017) and Vicente et al. (2020) also observed an increase in the amount of ammonia with increasing fish density. However, these studies did not report any significant relationship between the concentration of nitrite and nitrate in the water with stocking density, probably due to the use of organic carbon in their studies to regulate nitrogen in the bioflocs.

Table 2: Mean water quality parameters measured from the Nile tilapia nursery in a biofloc system when subjected to different stocking densities for 44 days.

Parameters	D200	D350	D500	D650	D800	Regression
Temperature (°C)	27.9 ± 0.1 (27.1–28.8)	27.9 ± 0.2 (27.1–29.0)	28.0 ± 0.1 (26.7–29.1)	27.9 ± 0.1 (27.2–28.8)	27.9 ± 0.2 (27.3–28.8)	y = 27.92
Dissolved oxygen (mg L^{-1})	7.1 ± 0.1 (6.0–7.6)	6.8 ± 0.2 (5.6–7.7)	6.4 ± 0.1 (5.0–7.6)	6.2 ± 0.1 (4.9–7.6)	6.2 ± 0.2 (4.7–7.5)	y = 7.29062–0.001498x. R^2 = 0.8576
pH	7.74 ± 0.01 (7.33–8.09)	7.61 ± 0.07 (6.80–8.05)	7.50 ± 0.07 (6.87–8.03)	7.42 ± 0.05 (6.95–7.98)	7.47 ± 0.04 (6.92–7.99)	y = 8.051–0.0018x+0.000001x^2. R^2 = 0.8604
TAN (mg L^{-1})	0.17 ± 0.04 (0.00–0.61)	0.21 ± 0.05 (0.00–0.71)	0.26 ± 0.02 (0.00–0.87)	0.25 ± 0.02 (0.00–0.85)	0.28 ± 0.02 (0.00–0.68)	y = 0.14183+0.000181x. R^2 = 0.6475
N-Nitrite (mg L^{-1})	0.12 ± 0.01 (0.00–0.40)	0.17 ± 0.01 (0.00–0.48)	0.31 ± 0.13 (0.00–0.88)	0.40 ± 0.07 (0.00–0.98)	0.50 ± 0.14 (0.00–1.70)	y =-0.03386+0.000669x. R^2 = 0.7731
N-Nitrate (mg L^{-1})	94 ± 13 (57–188)	121 ± 17 (60–197)	161 ± 25 (65–274)	162 ± 15 (64–315)	203 ± 27 (56–469)	Y = 61.883+0.17263x. R^2 = 0.7994
Alkalinity (mg $CaCO_3$ L^{-1})	73 ± 5 (55–92)	71 ± 5 (35–98)	70 ± 9 (39–116)	69 ± 3 (34–100)	74 ± 8 (45–120)	y = 71.38
Hardness (mg L^{-1})	174 ± 9 (120–230)	184 ± 12 (124–280)	207 ± 10 (136–310)	224 ± 14 (137–346)	227 ± 8 (136–336)	y = 154.23+0.097852x. R^2= 0.8105
Initial salinity (ppt)	0.74 ± 0.04	0.75 ± 0.03	0.74 ± 0.02	0.74 ± 0.03	0.75 ± 0.03	y = 0.7471
Final salinity (ppt)	1.18 ± 0.04	1.43 ± 0.05	1.69 ± 0.09	1.93 ± 0.02	2.21 ± 0.06	y = 0.83311+0.001711x. R^2 = 0.9845
Relative AC (%)	13.54 ± 0.01	13.71 ± 0.24	13.72 ± 0.27	13.85 ± 0.09	14.66 ± 0.13	y = 13.1047+0.001583x R^2 = 0.6452
Amount of base by biomass (g kg^{-1})	154 ± 1	160 ± 4	164 ± 5	165 ± 3	175 ± 5	y = 147.811+0.0315x. R^2 = 0.7806

Abbreviations: D200 – Stocking density of 200 fish m^{-3}. The other treatments followed variations of 350, 500, 600, and 800 fish m^{-3}. TAN – Total ammonia nitrogen. AC-alkalinity consumption.
Note: Data presented here are the mean ±standard deviation (minimum-maximum).

The use of organic carbon sources, such as molasses and sugar, promotes the use of ammonia-nitrogen by heterotrophic bacteria to form bacterial biomass instead of oxidizing ammonia to nitrate via nitrification. However, as per the heterotrophic route, one gram of ammonia generates 8.07 g of microbial biomass, indicating a much higher amount of solid generation, compared to the nitrification process (0.20 g) (Ebeling et al., 2006). Incidentally, Vicente et al. (2020) also reported an excess amount of total solids (> 1000 mg L^{-1}) at all stock densities (200 to 600 fish m^{-3} with weight ranging between 20 to 30 g per fish) evaluated in tilapia. This might be due to the use of sugar to neutralize the high concentration of ammonia present in a system even before the fish reached maturity. In this study, only the two highest stocking densities (D650 and D800) exceeded the value of 1000 mg L^{-1} of solids in the last week of the experiment (Figure 1).

The SS and TSS values showed a linear relationship with the tilapia density in BFT (Table 3). Several studies with tilapia have reported a wide variation in SS (0.5 to 65 mL L^{-1}) and TSS (200 to 1600 mg L^{-1}) values (Martins et al., 2017; Lima et al., 2018; Correa et al., 2020; Hisano et al., 2020; Martins et al., 2019; Durigon et al., 2020; Vicente et al., 2020). In this study, solids were managed to maintain average floc volume close to 30 mL L^{-1} and TSS to 600 mg L^{-1}, but it was difficult to maintain these levels after the experimental units reached biomasses above 6 kg m^{-3}. Over time there was a significant increase in the amount of TSS (Figure 1), and the only unit with the lowest density (D200) showed values below 600 mg L^{-1} throughout.

The total production of solids per experimental unit showed a positive linear relationship with increasing fish density. D800 units generated 4.4 times more solids than the D200, requiring rigorous system management. It was possible to identify the amount of solid generated by fish biomass and a quadratic relationship between this parameter and the stocking density of tilapia (Table 3, Figure 2).

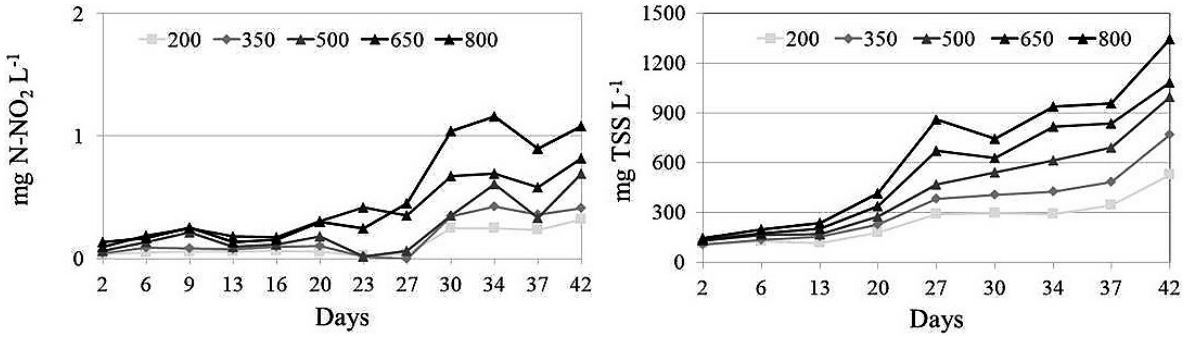

Figure 1: Nitrite concentration and total suspended solids (TSS) in the nursery water of Nile tilapia juveniles in a biofloc system when stocked with varied fish densities for 44 days.

Table 3: Solids parameters in the Nile tilapia nursery under a biofloc system packed with different stocking densities for 44 days.

Solids parameters	D200	D350	D500	D650	D800	Regression
Settling solids (mL)	14.2 ± 1.9 (2.5–30.0)	20.2 ± 1.2 (2.5–40.0)	21.4 ± 4.7 (3.0–60.0)	23.7 ± 3.5 (2.5–67.0)	28.6 ± 2.9 (4.0–110.0)	y = 10.8361+0.02153x. R^2 = 0.7302
Total suspended solids (mg L^{-1})	249 ± 11 (104–550)	336 ± 15 (102–813)	440 ± 19 (130–1155)	544 ± 8 (134–1180)	650 ± 24 (130–1560)	y = 107.74+0.6725x. R^2 = 0.9899
Total solids production (g EU^{-1})	63.9 ± 3.2	87.8 ± 4.2	133.6 ± 22.1	188.2 ± 16.8	281.8 ± 43.2	y = 0.310569x. R^2 = 0.8760
Solids produced by biomass (g kg $fish^{-1}$)	176.9 ± 8.7	151.5 ± 7.7	166.9 ± 33.1	179.3 ± 19.5	228.9 ± 34.9	y = 232.36–0.3784x +0.00047x^2. R^2= 0.6218

Abbreviations: D represents stocking density in m^{-3}, D200 – Stocking density of 200 fish m^{-3}, 350, 500, 600, and 800 represents the quantity of fishes m^{-3} in various experimental units.
Note: Data expressed as mean±standard deviation (minimum-maximum).

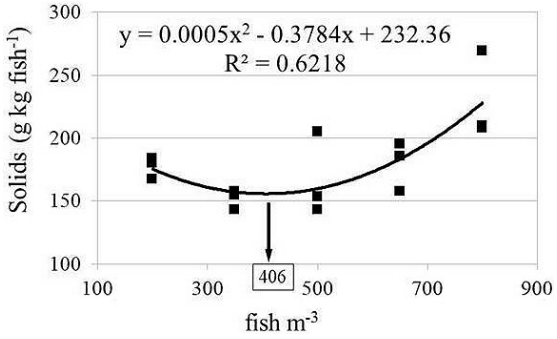

Figure 2: Total suspended solid production per kg of fish produced in the nursery for juveniles of Nile tilapia in a biofloc system subjected to different stocking densities for 44 days.

Figure 2 shows the density of 406 fish m^{-3} produced the lowest amount of solid per kg biomass of Nile tilapia juveniles. Solids are one of the limiting factors in BFT. The greater the amount of solids generated, the greater the damage to the growth phase of the tilapia (Manduca et al., 2020).

The mean pH values showed a quadratic relationship with the stocking density of the tilapia (Table 2), even though the pH remained at acceptable values for both the tilapia and the BFT (El-Sheriff; El-Feky, 2009; Martins et al., 2019). The pH decreased with increasing density (lowest in D200 and highest in D650). Haridas et al. (2017) and Vieira et al. (2019) also observed a reduction in the mean pH during tilapia cultivation with an increase in the density of juveniles in the BFT. This may be related to an increase in the CO_2 due to greater biofloc respiration and increased fish biomass in treatment units with higher stocking densities. However, this trend was not observed in the D800 treatment unit, probably due to the higher consumption of the base (Table 2). Both the relative consumption of the base (consumption of bicarbonate/ feed consumption) and the consumption of base by biomass

produced showed a positive linear relationship with stocking density (Table 2); we noticed a greater average increase in values mainly between D650 and D800. However, the values obtained for the relative consumption of base in all treatments are similar to those obtained in other studies using $NaHCO_3$as a carbon source for tilapia nurseries in BFT (Martins et al., 2017; Martins et al., 2019).

The total hardness of the water in tilapia juvenile nurseries showed a positive linear association with the stocking density (Table 2). The average values for total hardness varied from 128 to 152 mg L^{-1}, and it reached average values ranging from 220 to 321 mg L^{-1} toward the end of the experiment. This accumulation was probably due to the calcium and magnesium ions present in the feed since $NaHCO_3$was used in this study instead of hydrated lime [Ca $(OH)_2$] or calcium carbonate ($CaCO_3$) (Martins et al., 2017). Both the hardness (> 20 mg L^{-1}) and the hardness: alkalinity ratio (<5: 1) remained at the recommended limits preventing any impairment in the zootechnical performances of the tilapia juveniles (Cavalcante et al., 2012; Cavalcante et al., 2014).

Zootechnical performance

The negative linear relationship between tilapia growth and stocking density (Table 4) corroborates with results outlined in other similar studies (Haridas et al., 2017; Lima et al., 2018; Liu et al., 2018; Vicente et al., 2020). Brol et al. (2017) reported no significant differences in the growth pattern of Nile tilapia when stocking densities ranged from 400 to 800 fish m^{-3}; however, the final yield of the system was low (1.76 kg m^{-3}).

Yield and feed conversion showed a positive linear relationship with the stocking density (Table 4, Figure 3), similar to the findings reported by Liu et al. (2018). Unlike the studies conducted by Lima et al. (2018) and Vicente et al. (2020) that reported the maximum productivity of 15.27 kg m^{-3} and 9.91 kg m^{-3}, respectively, at an intermediate stocking density, we found the highest yield (12.85 kg m^{-3}) with the maximum density (D800), suggesting that it is possible to achieve better yields of tilapia juveniles in chemoautotrophic bioflocs. However, it is necessary to consider the increase in feed conversion rate, changes in water quality, increase in solids generation, and the increased production cost and management. Studies carried out by Lima et al. (2018), and Vicente et al. (2020) also showed higher feed conversions with increased density in their studies. However, the same was not observed in the works of Brol et al. (2017) and Haridas et al. (2017), where the density did not affect the feed conversion. In both these studies, the final yield of the systems was considered low (< 3.5 kg m^{-3}).

Figure 3 shows the effect of density in the tilapia nursery with BFT on the yield and feed conversion. It was observed that the increase in density affected the fish yield more than the feed conversion. Based on this, we recommend conducting further studies on commercial scales, allowing for a reliable production cost assessment and more precise determination of the optimum stocking density for the tilapia nursery in chemoautotrophic BFT.

Table 4: Zootechnical parameters of the Nile tilapia (0.66 ±0.17 g initial weight) reared in the nursery with a biofloc system for 44 days with varied stocking densities.

Parameters	D200	D350	D500	D650	D800	Regression
Final weight (g)	18.70 ± 0.13	17.21 ± 0.13	16.73 ± 0.50	16.93 ± 0.53	16.07 ± 0.41	y = 18.976–0.0037x. R^2 = 0.7171
SGR (%)	7.62 ± 0.01	7.43 ± 0.01	7.36 ± 0.05	7.35 ± 0.08	7.21 ± 0.02	y = 7.693–0.000598x. R^2 = 0.8500
DWG (g dia^{-1})	0.41 ± 0.01	0.38 ± 0.01	0.37 ± 0.01	0.37 ± 0.01	0.35 ± 0.01	y = 0.416621–0.000085x. R^2 = 0.7243
FCR	1.14 ± 0.01	1.17 ± 0.01	1.19 ± 0.03	1.19 ± 0.03	1.20 ± 0.03	y = 1.13032+0.0000925x. R^2= 0.4676
Yield (kg m^{-3})	3.74 ± 0.03	6.02 ± 0.04	8.36 ± 0.25	10.94 ± 0.27	12.85 ± 0.33	y = 0.66891+0.015432x. R^2 = 0.9953
Survival (%)	100 ± 0.0	100 ± 0.0	100 ± 0.0	99.50 ± 0.9	100 ± 0.0	y = 100
Uniformity (%)	65.0 ± 13.2	70.5 ± 6.6	57.3 ± 12.9	67.0 ± 5.4	64.2 ± 3.6	y = 66.51

Abbreviations: D200 – Stocking density of 200 fish m^{-3}. The numerical 350, 500, 600, and 800 represents the quantity of fishes m^{-3} in various experimental units. SGR – specific growth rate, DWG – daily weight gain, FCR – feed conversion rate.
Note: Data expressed as mean ± standard deviation (minimum-maximum).

In this study, survival was not influenced by density (Table 4) and remained above 98.5% at all stocking densities, corroborating with the findings reported by Haridas et al. (2017). However, in the studies by Lima et al. (2018) and Vicente et al. (2020), the highest densities (1250 fish m^{-3} and 600 fish m^{-3}, respectively) had low survival rates; it supports our earlier statement that critical biomass in their respective systems were achieved at intermediate densities. The growth data obtained in the present study at D800 are similar to or better than other studies (Alvarenga et al., 2018; Correa et al., 2020; Durigon et al., 2020).

Uniformity in growth was also not affected by the fish stocking density (Table 4). A large group of fish can reduce access to feed; therefore, increasing the heterogeneity in tilapia growth (Garcia et al., 2013). However, aggressive behavior can be reduced with soft lights as seen in BFT (El-Hawarry; Mohamed; Ibrahim, 2018; Gonçalves-de-Freitas et al., 2019), and the feed frequency used in this study (4x per day) could be the reasons behind the uniformity of growth in tilapia even at the high densities. However, some studies observed variations in growth with a high stocking density (Barbosa et al., 2006; Garcia et al., 2013).

Hematological analysis

Understanding the hematological profile of fish is an important indicator of animal health (Tavares-Dias et al., 2009).

In this work, no variation was observed in the number of erythrocytes and hematocrit (Table 5), indicating that the different fish densities, reared with BFT, did not alter blood homeostasis in tilapia. A recent study found no significant variations in tilapia erythrocyte counts when grown at different densities in bioflocs (Poli et al., 2021). However, they observed an increase in hematocrit in fish subjected to higher stocking densities. Stressful situations can lead to increased hematocrit due to changes in the electrolyte balance (Wendelaar Bonga, 1997).

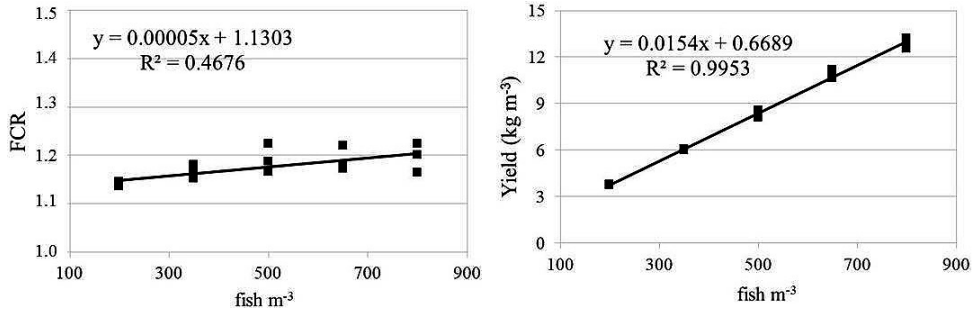

Figure 3: Feed conversion rate (FCR) and yield in the nursery where Nile tilapia juveniles were reared in a biofloc system for 44 days with different stocking densities.

Table 5: Hematological analysis of Nile tilapia juveniles reared in a biofloc system with different stocking densities for 44 days.

Hematological parameters	D200	D350	D500	D650	D800	Regression
Hematocrit (%)	27.5 ± 1.0	27.6 ± 2.5	26.8 ± 2.5	26.0 ± 2.1	29.7 ± 2.1	y = 27.52
Erythrocyte (x10^4 cells mL^{-1})	83.6 ± 23.7	97.0 ± 33.4	137.0 ± 58.4	128.2 ± 27.5	111.1 ± 77.0	y = 111.38
Thrombocyte (x10^3 cells mL^{-1})	5.22 ± 0.69	6.92 ± 3.58	5.86 ± 1.92	6.17 ± 2.75	4.88 ± 1.38	y = 5.81
Leukocyte (x10^3 cells mL^{-1})	127.5 ± 4.0	114.8 ± 18.1	112.3 ± 18.5	130.4 ± 17.0	92.25 ± 4.25	y = 115.47
Lymphocyte (%)	96.19 ± 2.25	97.31 ± 1.42	97.42 ± 1.66	95.00 ± 3.46	90.88 ± 7.63	y = 95.36
Monocyte (%)	0.00 ± 0.00	0.00 ± 0.00	0.19 ± 0.17	0.00 ± 00	0.00 ± 0.00	y = 0.04
Neutrophil (%)	0.11 ± 0.19	0.11 ± 0.19	0.31 ± 0.34	0.08 ± 0.14	0.00 ± 0.00	y = 0.12
Basophile (%)	0.00 ± 0.00	0.17 ± 0.29	0.39 ±0.35	0.50 ± 0.87	0.25 ± 0.25	y = 0.26
Eusinophile (%)	3.69 ± 2.20	2.42 ± 1.42	1.69 ± 1.25	4.42 ± 2.45	8.88 ± 7.38	y = 4.22

Abbreviations: D200 – Stocking density of 200 fish.m^{-3}. The other treatments followed variations of 350, 500, 600, and 800 fish m^{-3}.
Note: Data expressed as mean ±standard deviation (minimum-maximum).

Leukocytes play an important role in innate immunity and are considered an indicator of the health status of fish, as they are responsible for the immune response against pathogens (Batista-Neto et al., 2019). In the present study, no significant differences were detected in either total or differential leukocyte counts (Table 5). A recent study reported a significant reduction in total leukocytes with an increase in Nile tilapia density (Mahmoud et al., 2021).

In this study, total thrombocytes involved in inflammatory processes (Tavares-Dias; Moraes, 2007) were not affected by the stocking density (Table 5). However, Vicente et al. (2020) reported a significant increase of the thrombocytes in tilapia reared at a high stocking density of 600 fish m^{-3}.

CONCLUSIONS

It was possible to experiment with Nile tilapia nursery in chemoautotrophic BFT at densities up to 800 fish m^{-3} with final yields greater than 12 kg m^{-3} maintaining adequate water quality parameters for the Nile tilapia growth and survival. However, the highest efficiency concerning the production of solids and fish biomass produced occurred at the density of 406 fish m^{-3}.

AUTHOR CONTRIBUTION

Conceptual Idea: Silva, B.C.; Jatobá, A.; Methodology design: Silva, B.C.; Serafini, R.L.; Data collection: Silva, B.C.; Haluko, M.; Andrade, J. I. A.; Serafini, R.L.; Jatobá, A.; Data analysis and interpretation: Silva, B.C.; Andrade, J. I. A.; Jatobá, A.; Writing and editing: Silva, B.C.; Haluko, M.; Andrade, J. I. A.; Serafini, R.L.; Jatobá, A.

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