

NILE TILAPIA NURSERY IN A BIOFLOC SYSTEM: EVALUATION OF DIFFERENT STOCKING DENSITIES*

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ABSTRACT

This trial aimed to evaluate the growth performance and hematological parameters of Nile tilapia (*Oreochromis niloticus*) GIFT strain during nursery using different stock densities in a biofloc system. The experiment was conducted in circular tanks (400 L) with sexually reversed fish, weighing 6.74 ± 0.37 g, over a period of 35 days. Five treatments with three replicates each were used in a completely randomized design. The treatments were as follows: T1 (200 fishes m^{-3}); T2 (300 fishes m^{-3}); T3 (400 fishes m^{-3}); T4 (500 fishes m^{-3}), and T5 (600 fishes m^{-3}). The fishes were fed four times a day, following a feed table for this species, with adjustments according to fish biomass. The water quality parameters total ammoniacal nitrogen, and unionized ammonia showed a significant difference ($p < 0.05$) between the treatments with lower (T1) and higher stocking densities (T4, T5). Alkalinity was significantly higher in treatments with higher densities (T4 and T5). For hematological parameters, the number of thrombocytes was higher in T5. Hemoglobin concentration was significantly lower in T5 than in T3. The best productive indexes were found in T4, presenting final biomass of 9915.16 ± 14.80 g m^{-3} , apparent feed conversion rate of 1.11 ± 0.02 , mean survival of $95.75 \pm 0.75\%$, and daily mean weight gain of 0.43 ± 0.07 g. Overall, the Nile tilapia nursery in a biofloc system showed higher individual growth at densities up to 300 fishes m^{-3} and increased stocking density up to 500 fishes m^{-3} .

Keywords: *Oreochromis niloticus*; hematology; super-intensive system; BFT system.

BERÇÁRIO DE TILÁPIAS-DO-NILO EM SISTEMA DE BIOFLOCOS: AVALIAÇÃO DE DIFERENTES DENSIDADES DE ESTOCAGEM

RESUMO

O objetivo foi avaliar o desempenho zootécnico e os parâmetros hematológicos de tilápias-do-nylo (*Oreochromis niloticus*) linhagem GIFT, utilizando diferentes densidades de estocagem em sistema de criação em bioflocos. O experimento foi conduzido em tanques circulares (400 L), com peixes invertidos sexualmente, pesando $6,74 \pm 0,37$ g, em um período de 35 dias. Foi utilizado um delineamento inteiramente casualizado com cinco tratamentos e três repetições cada. Os tratamentos foram o T1 (200 peixes m^{-3}); T2 (300 peixes m^{-3}); T3 (400 peixes m^{-3}); T4 (500 peixes m^{-3}) e T5 (600 peixes m^{-3}). Os peixes foram arraçoados quatro vezes ao dia, seguindo a tabela de alimentação específica para a espécie, com ajustes pelas biometrias semanais. Os parâmetros de qualidade de água nitrogênio amoniacal total e amônia não-ionizada apresentaram diferença significativa ($p < 0,05$) entre os tratamentos com menor (T1) e maiores densidades de estocagem (T4, T5). A alcalinidade foi significativamente maior nos tratamentos com maiores densidades (T4 e T5). Dos parâmetros hematológicos, a quantidade de trombócitos foi mais elevada no T5 em relação aos demais tratamentos. A concentração de hemoglobina foi significativamente menor no T5 em relação ao T3. Os melhores índices produtivos foram encontrados no T4, apresentando biomassa final de $9915,16 \pm 14,80$ g m^{-3} , taxa de conversão alimentar aparente de $1,11 \pm 0,02$, sobrevivência média de $95,75 \pm 0,75\%$ e ganho médio diário de $0,43 \pm 0,07$ g. O berçário de tilápia-do-nylo em sistema de bioflocos apresentou maior crescimento individual em densidade de até 300 peixes m^{-3} e aumento de produtividade em densidade de estocagem de até 500 peixes m^{-3} .

Palavras chaves: *Oreochromis niloticus*; hematologia; sistema super-intensivo; BFT.

INTRODUCTION

Aquaculture is one of the fastest growing sectors of animal protein production in the world, at an annual rate of 8.3% since 1970 (FAO, 2017), contributing to the reduction of population poverty, hunger and malnutrition, generating economic

growth and guaranteeing natural resources (FAO, 2017). In 2014, world aquaculture production totaled 73.8 million tonnes, of which 49.8 million tonnes were fishes (67.4%) (FAO, 2017). Tilapia are the most produced fish group in the world (FAO, 2017), and Nile tilapia (*Oreochromis niloticus*) contributes 3.93 million tonnes (FAO, 2017). In Brazil, Nile tilapia is the most cultivated aquaculture species with 400,280 tonnes in 2018, which represents an increase of 11.9% over the previous year (PeixeBR, 2019). However, growth stumbles on the limited availability of natural resources (Verdegem, 2013), requiring the development of systems to increase production and productivity with less use of water, space and energy (Asche et al., 2008; FAO, 2017), yet with satisfactory economic returns.

In this context, intensive aquaculture systems are challenged by providing a favorable environment for high-density fish and shrimp production with little or no water exchange (Ray et al., 2010). Biofloc crops are increasingly common to meet such need (Avnimelech, 2006; Crab et al., 2007; De Schryver et al., 2008).

Biofloc Technology (BFT) involves a closed system for the rearing of aquatic organisms based on nutrient recycling and conversion into microbial flakes, which serve as endogenous natural food for production animals (Azim and Little, 2008). This system is driven by the principle of nutrient recycling through high carbon: nitrogen (C:N) ratio, stimulating the growth of heterotrophic bacteria, which transform ammonia into microbial bioflocs (Burford et al., 2003; Hari et al., 2004; Avnimelech, 2015). Biofloc cultivation occupies smaller areas of land and water volume (Moss et al., 2012). However, increasing stocking density and minimum or zero water renewal result in the accumulation of feed residues, excreta and toxic inorganic compounds (Burford et al., 2003), thus compromising water quality (Avnimelech, 2007) and, hence, health of the fish. Therefore, the accumulated sludge must be drained (Widanarni et al., 2012; Emerenciano et al., 2013).

Nile tilapias are capable of absorbing suspended biofloc, being adapted to high stocking densities (Avnimelech, 2011). Tilapias are able to efficiently utilize heterotrophic bacteria and are thus suitable for cultivation in biofloc systems (Choo and Caipang, 2015). Traditionally, tilapia crops in southern Brazil are concentrated in hot spells, in long and complete production cycles, with 0.5 g fingerling stocking and fishes over 600 g. This production model limits the competitiveness of the activity by having predefined fish stocks and supplies needed for commercialization (seasonalized). Biphasic production systems would involve fingerling cultivation in bioflocs, readying juveniles for production units. Such system might reduce cultivation time and minimize production seasonality, resulting in a more competitive activity.

Fish farming in closed systems, especially in biofloc cultivation, is a widespread practice in Brazil. However, initial investments are still high (Vilani et al., 2016), and practical results, whether economic, zootechnical or hematological, are still unknown for Nile tilapia fingerling cultivation in nursery systems. This calls for a better understanding of the zootechnical results of tilapia fingerling culture in biofloc systems. Therefore, this study aimed to evaluate the zootechnical performance and characterize the hematological parameters of juveniles of Nile tilapia *O. niloticus* in a biofloc system at different stocking densities.

MATERIAL AND METHODS

Biological material

For the study, 6,000 Nile tilapia (*O. niloticus*) GIFT strain, monosex, and sexually inverted, were obtained from the Fish Farming Unit of the Aquaculture and Fisheries Development Center (CEDAP), which is part of the Agricultural Research Company and Rural Extension from Santa Catarina (Epagri). They were transported in aerated boxes with constant dissolved oxygen to the Marine Shrimp Laboratory (LCM) / Federal University of Santa Catarina (UFSC), remaining in the box with constant aeration for 24 hours until distribution in the experimental units.

The experiment was approved by the Animal Use Ethics Committee (CEUA) (Protocol 7721291117).

Experimental design, experimental units and management

The experiment lasted 35 days and was conducted in a completely randomized design with five treatments and three replications, totaling 15 experimental units. The initial average weight was 6.74 ± 0.37 g.

The experimental units were 500 L circular polypropylene tanks (400 L of usable volume), independently allocated in an indoor room with 12/12 h (day / night) photoperiod maintained by artificial lighting.

The experiment was conducted using densities between 200 and 600 animals m^{-3} in the following treatments: T1: 200 fishes m^{-3} ; T2: 300 fishes m^{-3} ; T3: 400 fishes m^{-3} ; T4: 500 fishes m^{-3} and T5: 600 fishes m^{-3} . The animals remained for three days in the experimental units with previously fertilized water before the actual start of the experiment.

A circular-shaped microporous hose system arranged near the bottom of each experimental unit coupled to a blower aeration system (Ibram 7CV radial compressor) was used to keep the biofloc in suspension and the dissolved oxygen above 5.0 $mg\ L^{-1}$. Each experimental unit was individually equipped with a 500 watt thermostatically regulated heater to maintain water temperature at $28^{\circ}C$ throughout the experimental period. The salinity of the boxes was previously adjusted to 2.0 $g\ L^{-1}$, aiming to prevent possible N-nitrite poisoning and stress (Wuertz et al., 2013).

Throughout the experimental period, no water exchange took place, only replacement of the loss by evaporation and decantation.

Organic Fertilization

The water in the tanks was prepared three days before settlement using commercial tilapia feed (45% crude protein) and sugarcane molasses powder, maintaining a carbon-nitrogen ratio (C: N) of 20: 1 (Avnimelech, 2007).

Ammonia control throughout the experiment was performed by adding refined cane sugar as a source of organic carbon, applying a C: N 20: 1 ratio (Avnimelech, 1999) based on total ammonia nitrogen (NAT), and constantly maintaining 1.0 $mg\ L^{-1}$ of residual ammonia.

At the beginning of the experiment, the average temperature was $27.39 \pm 0.76^\circ\text{C}$; dissolved oxygen: $7.76 \pm 0.09 \text{ mg L}^{-1}$; alkalinity: $328.62 \pm 62.82 \text{ mg CaCO}_3 \text{ L}^{-1}$; Total Suspended Solids (TSS): $496.33 \pm 119 \text{ mg L}^{-1}$; sedimentable solids: $45.38 \pm 27.65 \text{ mL L}^{-1}$; pH: 7.99 ± 0.09 ; nitrite (N-NO₂): $0.01 \pm 0.008 \text{ mg L}^{-1}$; nitrate (N-NO₃): $4.50 \pm 5.11 \text{ mg L}^{-1}$; total ammonia nitrogen (TAN): $4.63 \pm 0.97 \text{ mg L}^{-1}$ and salinity: $1.62 \pm 0.34 \text{ g L}^{-1}$.

Food management

Fishes were fed according to the food table (Silva and Marchiori, 2018). The daily feeding rate was initially determined by the average weight of the populated animals and the average biomass of each treatment. Adjustments were made weekly by biometrics, water quality and total ammonia concentration (TAC), according to the consumption of each experimental unit. The animals were fed four times a day (08:00, 11:00, 14:00 and 17:00 h) with extruded 1.3 mm commercial feed containing 45% crude protein in the first 10 days and three times a day at 4 mm and 35% crude protein from day 11 until the end of the experiment. The daily feed supply was based on biomass, ranging from 3 to 6% per day.

Biometrics

The animals were individually weighed at the beginning of the experiment (0.01 g precision digital scale), and then weekly a sample of 10% of the population of each experimental unit was weighted to evaluate fish growth, make any adjustments in feed supplies, and perform macroscopic evaluations to verify any possible health problems. All animals were anesthetized with eugenol (50 mg L^{-1}) before the weighting procedures.

Water Quality

Throughout the experiment, no water exchange took place in the experimental units, only replacement of the evaporation loss and sludge settlements.

Temperature and dissolved oxygen were measured twice a day (7 am and 6 pm) in each experimental unit, using a portable digital oximeter (YSI Pro20). Results were expressed as the daily average of each treatment. The pH (Tecnal® pH-meter) and salinity (Eco-Sense YSI EC3 digital salinometer) were measured twice a week. Three times a week, before the first feeding, water was collected, and total ammonia nitrogen (TAN), nitrite (N-NO₂), nitrate (N-NO₃) and alkalinity (CaCO₃) analyses were performed. Whenever TAN exceeded 10 mg L^{-1} , feed was suspended for two subsequent feeding periods.

Management of the biofloc

On alternate days, sedimentable solids (SS) analyses were performed using one liter of water from the experiment, transferring to the Imhoff cone and reading the volume of sedimented material after 30 minutes, according to the method of APHA (2005), as adapted by Avnimelech (2015).

The volume of sedimentable solids for tilapia cultivation in biofloc systems should be maintained between 25 and 50 mL L^{-1} (Hargreaves,

2013). The volume of 50 mL L^{-1} was adopted as the limit, and excess was decanted through a 50 L volume conical settling tank connected to the cultivation tanks when this amount was achieved.

Total Suspended Solids (TSS) were analyzed once a week (APHA, 2005), using $0.6 \mu\text{m}$ porosity fiberglass filters (GF6 Macherey-Nagel).

For the growth and maintenance of the microbial community, sugar was added in order to obtain a carbon-nitrogen ratio (C: N) of 20: 1 (Avnimelech, 2015) daily.

Hematological analysis

Hematological analyses were performed at the end of the experiment, using three animals per repetition, nine per treatment, totaling 45 animals.

At the time of harvesting, the animals used for blood collection were anesthetized by immersion using eugenol (50 mg L^{-1}), weighed and measured individually. Subsequently, blood was collected by vaso-caudal puncture with previously heparinized sterile syringes for total and differential white blood cell count. After that, the animals were euthanized by spinal column concussion.

The samples were packaged and sent to the AQUOS / UFSC - Health of Aquatic Organisms Laboratory where they were processed in order to perform various analyses.

Blood was used for the confection and blood extensions, in duplicate, and stained with May Grunwald / Giemsa / Wright - MGGW (Ranzani-Paiva et al., 2013) for total and differential leukocyte count (white blood cells - WBC) and thrombocytes by the indirect method (Ishikawa et al., 2008).

One aliquot was used to determine the average hematocrit (Ranzani-Paiva et al., 2013) and the remainder to quantify the total number of erythrocytes (red blood cells - RBC) in a Neubauer chamber after 1: 200 dilution in Dacie's solution.

Hemoglobin concentration was analyzed by the cyanometahemoglobin method (Collier, 1944).

Hematimetric equations were used to determine mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) (Wintrobe, 1934).

Zootechnical Performance

The zootechnical performance of Nile tilapia was evaluated by weekly biometrics, weighing approximately 10% of the population of each experimental unit. At the end of the experiment, the animals of all experimental units were weighed and quantified to determine the zootechnical performance through the following variables and formulas: survival rate (%), average daily gain (GMD) (g) ($\% \text{ day}^{-1}$), final average weight (g), apparent feed conversion factor (FCA) (feed intake / fish weight gain), average productivity (kg m^{-3}) and specific growth rate (SGR) ($[(\ln \text{ final weight} - \ln \text{ initial weight}) / \text{cultivation time}] \times 100$).

Statistical Analysis

To assess normality and homoscedasticity, the Shapiro-Wilk and Levene test (Zar, 2010) were applied, respectively. Subsequently,

an analysis of variance (ANOVA) was performed, followed by Tukey's test (Zar, 2010), to compare means at a 5% significance level using Statistica® 6.0 software.

RESULTS

Water Quality

The oxygen dissolved in water was significantly lower ($p < 0.05$) in treatments T4 and T5 compared to water of treatment T1 (Table 1).

Total ammonia (TAN) and unionized ammonia in water were significantly higher ($p < 0.05$) in treatments T4 and T5 in relation to water in treatment T1.

Alkalinity was significantly higher ($p < 0.05$) in treatments T4 and T5 than in other treatments. The adjusted salinity showed no statistical difference among treatments ($p > 0.05$), ranging from 1.5 and 2.0 g L⁻¹ throughout the experiment (Table 1).

No statistical difference ($p > 0.05$) was observed in pH, N-nitrite, N-nitrate and TSS among treatments (Table 1).

Hematological parameters

Hemoglobin concentration was statistically lower ($p < 0.05$) in T5 fishes compared to T3 fishes. The number of thrombocytes was statistically higher ($p < 0.05$) at T5. Mean hematocrit, erythrocytes, MCV, MCHC, leukocytes, neutrophils, lymphocytes and monocytes showed no significant differences among treatments ($p \geq 0.05$) (Table 2).

Zootechnical Indices

The final average weight was statistically higher ($p < 0.05$) in treatment T1 (28.87 g) compared to treatments 3, 4, and 5 (21.65 g; 20.68 g and 17.87 g), respectively (Table 3). The average survival

Table 1. Physicochemical parameters of water quality of Nile tilapia (*Oreochromis niloticus*) cultivation in biofloc system at different stocking densities.

	Treatment					p value
	T1	T2	T3	T4	T5	
DO (mg L ⁻¹)	7.20±0.14 ^a	6.85±0.12 ^{ab}	6.82±0.17 ^{ab}	6.56±0.03 ^b	6.49±0.09 ^b	0.0008
Temperature (°C)	27.58±0.08	28.93±1.46	27.78±0.22	27.96±0.06	28.09±0.01	0.3604
TAN-N (mg L ⁻¹)	5.15±0.13 ^b	7.81±1.03 ^{ab}	6.97±1.34 ^{ab}	9.41±0.27 ^a	10.74±0.46 ^a	0.0009
N-NH ₃ (mg L ⁻¹)	0.58±0.03 ^b	0.83±0.16 ^{ab}	0.64±0.23 ^b	0.98±0.03 ^a	1.08±0.04 ^a	0.0339
N-NO ₂ (mg L ⁻¹)	0.04±0.01	0.16±0.13	0.07±0.04	0.10±0.06	0.08±0.02	0.4581
N-NO ₃ (mg L ⁻¹)	0.00±0.00	0.99±1.40	4.04±4.47	0.62±0.62	4.83±0.73	0.1526
Alkalinity (mg CaCO ₃ L ⁻¹)	212.22±18.50 ^b	255.33±12.42 ^b	232.81±64.06 ^b	332.44±1.11 ^a	331.67±5.00 ^a	<0.0001
pH	8.11±0.01	8.08±0.03	7.92±0.28	8.06±0.03	8.03±0.01	0.0601
TSS (mg L ⁻¹)	1066.33±94.28	1076.67±221.09	1611.67±185.70	1301.20±21.20	1163.90±181.90	0.3582
Salinity (g L ⁻¹)	1.96±0.55	1.64±0.12	1.91±0.41	1.64±0.02	1.59±0.00	0.0713

T1: 200 fishes m⁻³; T2: 300 fishes m⁻³; T3: 400 fishes m⁻³; T4: 500 fishes m⁻³; T5: 600 fishes m⁻³. DO: dissolved oxygen; TAN: total ammonia nitrogen; N-NH₃: unionized ammonia; N-NO₂: nitrite; N-NO₃: nitrate. TSS: total suspended solids (TSS). Values are expressed as mean ± standard deviation. Means followed by different letters on the same line indicate significant difference by Tukey test ($p < 0.05$).

Table 2. Hematological parameters of Nile tilapia (*Oreochromis niloticus*) cultivated in biofloc system at different stocking densities.

	Treatment					P value
	T1	T2	T3	T4	T5	
Hematocrit (%)	30.78±4.80	27.11±3.11	28.11±6.94	26.00±7.68	29.17±3.48	0.5525
Erythrocyte (x10 ⁶ µL ⁻¹)	2.51±0.50	1.95±0.17	2.22±0.30	2.31±0.39	2.27±0.32	0.0575
Hemoglobin (g dL ⁻¹)	12.81±2.25 ^{ab}	10.95±1.24 ^{ab}	13.85±2.95 ^a	11.37±2.91 ^{ab}	9.69±2.60 ^b	0.0321
MCV (fL)	126.52±28.88	139.60±17.02	127.84±30.41	110.62±22.22	129.74±16.94	0.3609
MCHC (g dL ⁻¹)	42.07±7.36	40.92±6.74	54.62±25.84	47.89±17.64	33.15±7.88	0.1527
Thrombocytes (x10 ³ µL ⁻¹)	16.90±6.26 ^b	16.05±11.79 ^b	15.64±9.40 ^b	7.28±4.84 ^b	39.11±16.40 ^a	0.0002
Leukocytes (x10 ³ µL ⁻¹)	71.49±26.74	53.40±24.43	50.95±18.15	35.24±98.70	52.09±27.42	0.0988
Neutrophils (x10 ³ µL ⁻¹)	4.56±39.19	2.64±18.22	3.61±2.12	4.89±2.09	5.88±5.64	0.4650
Lymphocytes (x10 ³ µL ⁻¹)	118.49±22.09	91.78±7.44	102.51±16.70	105.98±18.03	103.49±19.58	0.0702
Monocytes (x10 ³ µL ⁻¹)	2.68±12.91	3.09±2.01	4.83±4.81	4.64±3.78	4.38±3.54	0.6404

T1: 200 fishes m⁻³; T2: 300 fishes m⁻³; T3: 400 fishes m⁻³; T4: 500 fishes m⁻³; T5: 600 fishes m⁻³. MCV (mean corpuscular volume); MCHC (mean corpuscular hemoglobin concentration). Values are expressed as mean ± standard deviation. Means followed by different letters on the same line indicate significant difference by Tukey test ($p < 0.05$).

Table 3. Zootechnical performance of Nile tilapia (*Oreochromis niloticus*) cultivated in biofloc system under different stocking densities.

	Treatment					p value
	T1	T2	T3	T4	T5	
FW (g)	28.87±0.67 ^a	25.58±1.86 ^{ab}	21.65±3.91 ^{bc}	20.68±0.22 ^{bc}	17.87±0.16 ^c	0.0001
Survival (%)	95.83±0.59 ^b	94.45±2.08 ^b	90.41±6.66 ^{ab}	95.75±0.75 ^b	78.13±6.05 ^a	0.0054
FCR	1.02±0.04 ^a	1.04±0.10 ^a	1.21±0.26 ^b	1.11±0.02 ^{ab}	1.51±0.26 ^c	0.0145
SGR (% day⁻¹)	3.90±0.08 ^a	3.76±0.13 ^a	3.39±0.46 ^{ab}	3.23±0.01 ^{ab}	2.95±0.04 ^b	0.0080
Yield (kg m⁻³)	5.53±0.15 ^c	7.25±0.69 ^{bc}	7.85±0.174 ^{ab}	9.91±0.14 ^a	8.38±0.99 ^{ab}	0.0012
WG (g)	17.22±0.27 ^a	15.25±0.61 ^{ab}	12.75±2.39 ^{bc}	9.93±0.22 ^c	9.83±0.56 ^c	0.0002
DWG (g day⁻¹ fish⁻¹)	0.61±0.02 ^a	0.53±0.05 ^{ab}	0.43±0.12 ^b	0.43±0.04 ^b	0.37±0.07 ^b	0.0080

T1: 200 fishes m⁻³; T2: 300 fishes m⁻³; T3: 400 fishes m⁻³; T4: 500 fishes m⁻³; T5: 600 fishes m⁻³. FW: Final weight, FCR: Feed conversion rate, SGR: Specific growth rate, WG: Weight gain, DWG: Daily weight gain. Values are expressed as mean ± standard deviation. Means followed by different letters on the same line indicate significant difference by Tukey test (p<0.05).

was lower (p<0.05) in T5 and T3, respectively, compared to the other treatments (Table 3).

The feed conversion factor was higher in T5 when compared to T1 and T2. Specific growth rate (SGR) was statistically higher (p<0.05) in treatments 1 and 2 compared to treatment 5 (Table 3).

In addition, the yield was higher (p<0.05) in treatment 4 compared to treatment 1 and 2. The average daily weight gain in the experimental period was statistically higher (p<0.05) in treatment 1, fishes compared to T3, T4 and T5 treatments (Table 3).

DISCUSSION

The lower dissolved oxygen in treatments with higher densities (T4 and T5) may be attributed to the higher oxygen consumption owing to higher fish biomass in addition to higher feed and sugar intake. However, the dissolved oxygen above 6.0 mg L⁻¹ remained in the ideal range for the growth of Nile tilapia (*Oreochromis niloticus*), according to Santos et al. (2013). Similar values of oxygen dissolved were reported by Correa et al. (2020) in the rearing of Nile tilapia juveniles in a biofloc system employing periods of feed deprivation. Zaki et al. (2020) also reported significantly higher (p<0.05) dissolved oxygen values in a biofloc system of Nile tilapia at the lower stocking density.

High dissolved oxygen (5-8 mg L⁻¹) is critical to maintain respiration of crop species, as well as microorganisms that make up the composition of suspended flakes (Hargreaves, 2013). Tilapia might tolerate lower oxygen levels (0.5 mg L⁻¹) (Popma and Lovshin, 1996), or they might even use surface air when dissolved oxygen from water is zero. Nonetheless, it is appropriate to maintain levels above 2-3 mg L⁻¹ to limit stress in animals (Popma and Lovshin, 1996) with ideal values above 4 mg L⁻¹ for BFT systems (Avnimelech et al., 2012).

The pH showed no significant difference among treatments (p>0.05), presenting light oscillations within the comfort range for *O. niloticus* tilapia (Azim and Little, 2008; El-Sherif and El-Feky, 2009; Widanarni et al., 2012). *Oreochromis niloticus* tilapia can tolerate pH ranges between 4 and 11 (Balarin and Hatton, 1979);

however, they present a better performance at neutral or slightly alkaline pH (Popma and Lovshin, 1996). The ideal pH for BFT systems ranges from 7 to 9, but it can oscillate throughout the day through the nitrification process (Avnimelech et al., 2012).

Prior to the start of the experiment, salt (sodium chloride) was added to the water in order to prevent possible nitrite stress, based on recommendations by Wuertz et al. (2013), and previously applied in studies by Luo et al. (2014) and Day et al. (2016).

Total ammoniacal nitrogen (TAN) concentrations were higher in treatments with higher stocking densities, especially in T4 and T5, probably as a consequence of the higher feed intake in these treatments during the experimental period, resulting in a higher concentration of nitrogen metabolites and even causing occasional mortality in the 600 fishes per m³ treatment. Although unionized ammonia (N-NH₃) was significantly higher in the treatments with higher densities, values were above the recommended for tilapia cultivation in all treatments, suggesting that it may have had a negative influence on the yield.

In order to avoid harming the fishes, the level of unionized ammonia (N-NH₃) should be below 0.05 mg L⁻¹ (Sá, 2012). Concentrations of 7.40 ± 0.01 mg L⁻¹ of total ammonia at pH 8.0 may cause 50% animal mortality in 48 hours (Karasu and Köksal, 2005). The higher levels of nitrogen compounds also caused a greater supply of organic carbon sources and, consequently, lower oxygen levels and higher alkalinity levels. However, in all treatments, alkalinity remained above 100 mg L⁻¹, thus not theoretically representing a limiting factor for nitrification and ammonia sequestration by heterotrophic bacteria (Avnimelech, 2015).

In a study with tilapia, Thurston et al. (1986) observed no loss in the zootechnical performance of fishes exposed to 0.44 mg L⁻¹ of unionized ammonia, while, at the same time, a negative effect on growth and survival was observed when exposed to 0.91 mg L⁻¹. In the present study, in experimental units where TAN exceeded 10 mg L⁻¹, coinciding with the reduced feed intake at these times, especially in treatments with densities of 500 and 600 tilapias per m³, it may have negatively influenced the growth rate, as corroborated by El-Shafai et al. (2004).

Nitrogen compounds are considered the main limiting factor for the survival of cultivated aquaculture organisms (Barbieri, 2010; Xian et al., 2011; Santacruz-Reyes and Chien, 2012), after dissolved oxygen. These compounds present in water, especially total ammonia nitrogen (TAN) ($\text{NH}_3 + \text{NH}_4$), are originated from unconsumed feed and from protein catabolism (El-Sayed, 2006; Crab et al., 2007). It is well known that unionized ammonia (N-NH_3) is toxic to cultivated organisms (El-Shafai et al., 2004). Ammonia is oxidized to nitrite by nitrifying bacteria, a highly toxic compound, and then to nitrate, a less toxic compound to animals (Avnimelech, 1999). In BFT, an alternative pathway is the removal or recycling of these compounds by the predominance of heterotrophic bacteria.

Nitrogen compounds may cause histological and hematological damage, affecting liver and gill function, such as tissue hypoxia, decreasing fish growth (Wajsbrodt et al., 1993). Dietary protein digestibility and energy source might be affected by unionized ammonia (Hargreaves and Kucuk, 2001). The biochemistry of protein, carbohydrate and energy derived from fat is compromised by the presence of ammonia, resulting in a reduction of up to 68% in the energy production rate (Zieve, 1966), which is necessary for ammonia detoxification, thus contributing to a reduction in growth rate.

N-nitrite and N-nitrate concentration remained low throughout the experimental period. Nitrite peaks are observed at the beginning of the nitrification process and when aeration is insufficient (Avnimelech et al., 2012). Nitrification was limited, making it difficult to reduce the concentration of TAN, likely a result of the short experimental time. Similar results were found in other studies (Liu, et al., 2014). Nitrite concentrations above 5.0 mg L^{-1} begin to result in mortality (Rakocy, 1989); therefore, ideal levels for optimal production should be below 1.0 mg L^{-1} (Avnimelech et al., 2012).

Total suspended solids (TSS) were higher than 1000 mg L^{-1} , which is the recommended upper limit (Avnimelech, 2006; Hargreaves, 2013) in all treatments throughout the experiment, limiting fish growth (Azim and Little, 2008; Long et al., 2015).

Hemoglobin concentration was lower in T5 compared to T3. However, in all treatments, the values found were above those reported for the species (Brum et al., 2017; Owatari et al., 2019). The main function of hemoglobin, a main health parameter of fishes, is carrying oxygen. Decreased hemoglobin concentration may result from uncontrolled stress-causing environmental conditions (Daneshvar et al., 2012). A noticeable decrease in hemoglobin and hematocrit rates in contaminated environments compared to normal ones has been reported (Summarwar, 2012). In the present study, the low values may be related to toxic ammonia concentrations.

The lymphocytes amount did not differ significantly ($p > 0.05$). However, they were elevated in all treatments, according to other studies (Ghiraldelli et al., 2006; Brum et al., 2017; Owatari et al., 2019). Lymphocytes are the largest defense cells under normal physiological conditions (Martins et al., 2004; Ranzani-Paiva and Silva-Souza, 2004). It is suggested that the lymphocytosis found in this study may be related to the immunomodulator potential of the biofloc.

Thrombocytes were higher in T5, and they were much higher than those found in other studies (Ghiraldelli et al., 2006; Brum et al., 2017; Owatari et al., 2018; Owatari et al., 2019). Thrombocytes play an important role in blood clotting and in general mechanisms of inflammatory processes (Tavares-Dias and Moraes, 2007; Kayode and Shamusideen, 2010). The higher number of thrombocytes suggests greater recruitment of their reserve compartments, contributing to organic defense mechanisms (Tavares-Dias et al., 1999). The high number may be indicative of a eutrophic environment (Ghiraldelli et al., 2006).

The average hematocrit was within the desirable range for the species in the early stages of cultivation, in clear water, with an average weight of $1.84 \pm 0.52 \text{ g}$ (Brum et al., 2017), and in studies with juvenile animals between 50 and 60 g (Owatari et al., 2018, 2019). Changes in hematocrit (hemoconcentrated or hemodiluted) may be related to stress (Morgan et al., 1997). Decreased percentage of hematocrit, hemoglobin and erythrocytes may be caused by an infection resulting in red cell lysis (Tamamduhuri et al., 2016).

The increase in the number of thrombocytes observed in T5 may be related to red cell lysis or may indicate a process of cell phagocytosis, due to the stress caused by the high stocking density, and also due to the several microorganisms present in the biofloc system (Correa et al., 2020; Durigon et al., 2020). However, in our study, no sign of any infection was observed in the animals.

MCHC was within the standards already found by other authors (Brum et al., 2017; Owatari et al., 2018, 2019).

Leukocytes in T1, even though absent statistical difference, were above the values of other studies with tilapia cultivated in excavated tanks and clear water (Ghiraldelli et al., 2006; Brum et al., 2017; Osman et al., 2018). Many stressors can cause leukocyte cells to increase (Biswas et al., 2004). It is suggested that the lower stocking density contributed to the lower environmental stress, improving the immune defense capacity in T1 compared to the others, and possibly reflecting better production rates, such as weight gain, feed conversion factor and survival.

Neutrophil numbers were within the values found in other trials (Ghiraldelli et al., 2006; Brum et al., 2017), and monocyte numbers were similar to those found by Owatari et al. (2018).

The higher average final weight and specific growth rate at T1 compared to T5 may be related to the lower stocking density in that treatment, which provided a better environmental quality with lower concentration of nitrogen compounds and other metabolite residues (Stickney, 2005) and, consequently, lower stress, allowing greater individual growth of fishes.

Survival was affected by crop density. An inverse relationship between survival and stocking density was evidenced in other studies in BFT (Widanarni et al., 2012) and recirculation (Suresh and Lin, 1992). The greater TAN concentration in the higher density treatments, especially in T5, contributed to the poor performance in this treatment, culminating in punctual mortalities.

The highest feed conversion ratio (FCR) in treatment T5 was a direct reflection of poor survival. However, the high concentration of nitrogen compounds may contribute to the increase of feed conversion.

Unionized ammonia concentrations above 0.144 mg L⁻¹ also may increase the feed conversion factor, according to El-Shafai et al. (2004).

The yield (final biomass) was higher ($p < 0.05$) in T4 as a consequence of storage density and satisfactory average survival percentage in this treatment, even though the average daily growth rates and weight gain in the period were lower.

The higher weight gain and average daily weight seen in the T1 treatment can most likely be attributed to the lower stocking density, as stated by Gall and Bakar (1999).

In general, the productive performance was affected by the concentration of nitrogen metabolites in the system, which is inversely related to stocking density (Avnimelech and Kochba, 2009; Widanarni et al., 2012).

The data obtained in the present study suggest that higher fish density results in higher production, yet lower survival and growth.

CONCLUSION

Nile tilapia nursery can be performed in a biofloc system; however, water quality parameters were affected by higher crop density, mainly between 500 and 600 fishes per m³, consequently affecting hematological and zootechnical indexes. The best growth rate was obtained at densities up to 300 fishes per m³, while the highest yield was observed at densities up to 500 fishes per m³.

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