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THE INTENSIVE CULTURE OF NILE TILAPIA SUPPLEMENTED WITH THE MICROALGAE *Chlorella vulgaris* IN A BIOFLOC SYSTEM

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ABSTRACT

The aim of this study was to evaluate the performance of Nile tilapia fingerlings cultured in biofloc technology using different inoculation densities of *Chlorella vulgaris*. The experimental design was completely randomized with biofloc system and four densities of *Chlorella vulgaris* (0, 2.5, 5 and 10 x 10^4 cell mL^{-1}), each with four replications. The study lasted 63 days and was carried out in tanks with a working volume of 40L, at a stocking density of 10 fish per experimental unit and a mean initial weight of approximately 1.86 g. The water quality variables showed no significant difference between treatments, especially total ammonia nitrogen and nitrite nitrogen, which were within acceptable levels for culture of the species. The variables of zootechnical performance were not affected by the different inoculation densities of the microalgae, achieving a final mean weight of approximately 21 g for all treatments, and survival rates greater than 80%. The weekly inoculation densities of the microalgae *Chlorella vulgaris* therefore had no influence on the growth of tilapia fingerlings cultured in a biofloc system.

Key words: fingerlings culture; biofloc system; live food; phytoplankton; hematological indices.

CULTIVO INTENSIVO DA TILÁPIA DO NILO SUPLEMENTADO COM MICROALGA *Chlorella vulgaris* EM SISTEMA DE BIOFLOCOS

RESUMO

O presente trabalho teve como objetivo avaliar o desempenho de alevinos de tilápia do Nilo, cultivados em tecnologia de bioflocos, utilizando diferentes densidades de inoculação de *Chlorella vulgaris*. O delineamento experimental foi inteiramente casualizado, envolvendo o cultivo em sistema de bioflocos e quatro densidades de *Chlorella vulgaris* (0; 2,5; 5 e 10x10^4 cel mL^{-1}), com quatro repetições cada. O trabalho teve duração de 63 dias, sendo realizado em caixas com 40L de volume útil, densidade de estocagem de 10 peixes por unidade experimental e peso médio inicial de aproximadamente 1,86 g. As variáveis de qualidade de água não apresentaram diferença significativa entre os tratamentos, principalmente o nitrogênio da amônia total e do nitrito, que estiveram dentro do nível aceitável para o cultivo da espécie. As variáveis de desempenho zootécnico não foram afetadas pelas diferentes densidades de inoculação da microalga, obtendo peso médio final de aproximadamente 21 g para os tratamentos e taxas de sobrevivência superiores a 80%. Portanto para estas densidades de inoculação da microalga *Chlorella vulgaris*, com frequência semanal, não apresentaram influência no crescimento de alevinos de tilápia cultivada com bioflocos.

Palavras-chave: alevinagem; sistema de bioflocos; alimento vivo; fitoplâncton; índices hematológicos.

INTRODUCTION

In recent years, Nile tilapia (*Oreochromis niloticus*) has become one of the most cultured species in Brazil and around the world. Its strong presence in Brazilian aquaculture is due to the advantages and characteristics presented by the specie, such as the intake of a large variety of food, including phytoplankton, particles and debris suspended in the water column (Dempster et al., 1995; Azim et al., 2003; Bosisio et al., 2017). Due to these peculiarities, the specie exhibits the possibility for production in different culture systems, including a super-intensive biofloc system (Azim and Little, 2008; Avnimelech, 2009; Samocha et al., 2017).

The biofloc system has been widely studied because of its benefits compared to the traditional system, such as the possibility of increasing stocking densities, as well as reusing the culture water over several production cycles (Avnimelech, 2009; Samocha et al., 2017). The possibility of reusing the water is mainly due to the presence of the chemotrophic and heterotrophic bacteria that transform nitrogenous compounds into microbial protein, allowing control of the water quality in relation to these compounds (Wasielesky et al., 2006; Krummenauer et al., 2012; Emerenciano et al., 2013; Samocha et al., 2017).

The flocculated particles (bioflocs) formed in this system may serve as supplementary feed for the individuals being cultured, however their nutritional quality depends on the conditions of each production system (Burford et al., 2004; Wasielesky et al., 2006; Avnimelech, 2009; Samocha et al., 2017). The dry weight of these aggregates may reach high levels of crude protein, between 35 and 38%; however, they have a low percentage of lipids, around 1-5% (Tacon et al., 2002; Azim and Little, 2008). As an alternative, microorganisms can be added to improve the nutritional quality of the produced flakes, such as microalgae, which are naturally composed of high concentrations of lipids, which can reach 20-50% of their dry weight (Brennan and Owende, 2010), besides having proteins and carbohydrates in their composition (Chacón-Lee and González-Mariño, 2010). These microscopic beings are known for their different uses; however, in aquaculture their main use is as live food (Juarez et al., 2010).

The genus *Chlorella* is widely used in the nutrition of aquatic organisms, mainly because it is fast growing and tolerant to various culture conditions (Lourenço, 2006) and because of its nutritional content (Moronta et al., 2006), which in dry weight can vary from 5 to 40% lipids and 10% minerals and vitamins under optimal culture conditions, 42 to 58% protein, and 12 to 55% carbohydrates under nitrogen limitation (Kay and Barton, 1991; Becker, 1994; Brányiková et al., 2007).

However, research on the addition of microalgae in the culture of tilapia fingerlings in a biofloc system is still unknown in the early stages. The aim of this work therefore, was to evaluate the influence of the microalgae *Chlorella vulgaris* on the performance variables of tilapia cultured in bioflocs during stocking.

MATERIAL AND METHODS

The experiment was carried out at the Aquaculture Production Systems Laboratory (LAPAq) at the Aquaculture Station of the Federal Rural University of Pernambuco (UFRPE), where for 63 days the zootechnical performance of tilapia (*O. niloticus*) was evaluated using a completely random experimental design with four treatments (BFT, $BFT_{2.5}$, BFT_5 and BFT_{10}), including a biofloc system and different inoculation densities of *Chlorella vulgaris* (Control, $2.5x10^4$, $5x10^4$ and $10x10^4$ cell mL^{-1}) with four replications. The experimental units, located in a closed environment, covered with screens to avoid the fish escaping, and with constant aeration, consisted of rectangular tanks with a working volume of 40 liters (0.2 m^2), which were supplied with 27L (67.5%) of previously chlorinated fresh water (10 ppm active chlorine), dechlorinated by constant aeration for 24 hours, and 13L of biofloc (32.5%) from the growth phase of tilapia culture, so as to start the experiment with 7 mL L^{-1} settleable solids. Sugar-cane molasses was used as the organic carbon source, calculated to maintain a C to N ratio of 6:1, and then added to the system based on the amount of total ammonia nitrogen (TAN) in the water, whenever TAN levels were greater than 0.7 mg L^{-1}.

The chlorophyte *Chlorella vulgaris* was grown in the Live Food Production Laboratory (LAPAVI) of the Department of Fisheries and Aquaculture, enriched in Provasoli culture medium (1975) and B-complex vitamins, using 1.0 mL L^{-1} of each solution. The microalgae was kept in fresh water at a pH of 7.9, a temperature of 23.0 ± 1°C and a light intensity of ~2000 lux under a photoperiod of 24h light, and was inoculated every 7 days in the experimental units during the exponential phase of the growth curve, to guarantee that the physiological state of the cells and the high level of nutritional quality remained constant. In order to inoculate the specific cellular densities for each treatment, the density of the microalgae was counted with the aid of a Neubauer chamber and optical microscope, and the volume to be added to each experimental unit was then calculated.

Water quality

Water quality was monitored by observing the physicochemical variables of temperature (ºC), dissolved oxygen (mg L^{-1}) and pH, which were measured daily (at 8:00 A.M. and 4:00 P.M.) using the YSI 556 MPS multiparameter (YSI Incorporation, Ohio, USA). Water samples were collected weekly from each tank to determine the levels of total ammonia nitrogen (TAN), nitrite-N (NO_2-N) and total alkalinity, and every two weeks to analyze the nitrate (NO_3) and orthophosphate (PO_4). In order to maintain total alkalinity around 150 mg L^{-1} $CaCO_3$, sodium bicarbonate was used when necessary (Samocha, et al., 2017), with a total of 145.8g for treatment BFT, 132.2g for $BFT_{2.5}$, 139.4g for BFT_5 and 129.2g for BFT_{10}. The water was exchanged the minimum number of times necessary for controlling the volume of solids and replacing losses through evaporation every week. The nitrogen compounds were measured using the HACH TNT 830 (salicylate), 8507 (diazotization) and 8539 (cadmium reduction) methods for NAT, NO_2-N and NO_3 respectively; the orthophosphate concentration was measured using the PhosVer®3 8048 (ascorbic acid) method. The samples were read using the HACH DR 2800 digital spectrophotometer (Hach Company, Colorado, USA) and the concentration of total alkalinity was determined by volumetric titration (APHA, 1995).

To quantify the increase in microbial flocs throughout the culture period, the total suspended solids were analyzed every two weeks as per APHA (1995), and the sedimentable solids (mL L^{-1}) were measured once a week using Imhoff cones, with a 1L sample being collected from each experimental unit after 30 minutes decantation and rest (Avnimelech, 2009). Sedimentation tanks were installed as needed to maintain the volume of sedimentable solids at 15mL L^{-1}.

Zootechnical performance

The fingerlings of *O. niloticus* were obtained from a commercial fish farm and acclimated in a concrete tank, measuring 5 x 3 x 0.4 m, maintained with fresh water until they reached a weight of 1.87 ± 0.06g and fed on Guabi fish feed (1-2mm), containing 45% crude protein, 28% carbohydrate, 15% mineral material, 8% ether extract and 4% crude fiber. During the acclimation period, 70% of the water volume of the culture unit was renewed daily. The fingerlings were later counted, weighed and stocked in the experimental units at a density of 10 individuals (250 fish m^{-3}) per tank. The fish were fed on Presence (3-4 mm) extruded commercial feed, containing 36% crude protein, 37% carbohydrate, 14% mineral material, 8% ether extract and 5% crude fiber, four times a day, at 8:00 A.M., 11:00 A.M., 2:00 P.M. and 5:00 P.M., until the apparent satiation of the animals (*ad libitum*). At the end of each day throughout the experimental period, the unconsumed feed was weighed to determine the daily consumption.

At the end of the culture period, the fish from each experimental unit were counted and weighed to evaluate yield; it was thereby possible to calculate the weight gain ($WG = W_f - Wi$), daily weight gain ($DWG = WG.t\ (d)^{-1}$), feed conversion ratio (amount of feed offered.BG^{-1}), specific growth rate ($ln\ W_g$-$ln\ W_i$)*100.t^{-1}), biomass gain ($BG = B_f$-B_i), productivity (B_f.$volume^{-1}$) and survival ($(N_i$-$N_f)$*100.N_i^{-1}).

Hematological analysis

At the end of the culture period, five fish from each experimental unit were desensitized in eugenol solution (1:1000) to collect blood to analyze the hematological parameters. About 0.5 mL of blood from each fish was collected by caudal puncture using a heparinized syringe. About 0.3 mL of the sample, which was used for the mean erythrocyte count in a Neubauer chamber ($x10^6 \mu L^{-1}$), was packed in an anticoagulant tube and then diluted in 0.65% saline solution (Azevedo et al., 2006) in the proportion of 1:200. To determine corpuscular volume, the hematocrit technique was used (Goldenfarb et al., 1971), in which the microcapillary tube was filled with a blood sample at 2.3^{-1} of its total volume, centrifuged at 12000 rpm for 30 seconds and then measured on a calibration chart. The Mean Corpuscular Volume (MCV), which allows the volume of erythrocytes to be determined, was calculated using the formula: MCV = (Hematocrit x 10).number of $erythrocytes^{-1}$ ($x10^6 \mu L^{-1}$), described by Wintrobe (1934).

Statistical analysis

First the D'Agostino-Pearson normality test was carried out on the data for temperature, pH and dissolved oxygen, the Shapiro-Wilk test on the other variables, and Cochran's test for homoscedasticity at a significance level of 5%. The results for sedimentable solids, alkalinity and total ammonia nitrogen were transformed by cosine(Yi), sin(Yi) and Ln(Yi) respectively. When normality of the sample and homogeneity of the variances were found, Analysis of Variance (ANOVA) was applied to the culture variables. The Analysis of Variance test for samples repeated over time was applied to the variables of water quality. When a statistical difference was found, Tukey's mean-value comparison test was performed at a significance level of 5%. The Kruskal-Wallis test was applied to the data for nitrate, temperature (afternoon), pH and dissolved oxygen, as these did not present a parametric distribution. The statistical analysis was carried out using the SysEAPRO v1.0 software. The correlation between the different microalgae inoculation densities and the hematological variables was investigated by means of the Pearson correlation, calculated with the R 3.4.4 software.

RESULTS

Water quality

The mean values for water temperature, dissolved oxygen, pH, alkalinity, total ammonia nitrogen, nitrite nitrogen, nitrate, orthophosphate, sedimentable solids (SS) and total suspended solids (TSS) monitored during the experimental period displayed no significant differences between treatments ($p<0.05$), and are shown in Table 1; these represent the effect on the water quality variables of the different concentrations of *Chlorella vulgaris* inoculated into the experimental units in the culture of tilapia (*O. niloticus*) in a biofloc system during stocking.

A variation of 2 °C could be seen in temperature throughout the day, varying between approximately 26 °C in the morning and 28 °C in the afternoon. There was a variation in the dissolved oxygen of 3.85 to 8.50 mg L^{-1}, with mean values of 6.49 and 5.89 mg L^{-1} during the morning and afternoon respectively, whereas the pH varied between 8.25 in the morning and 8:14 in the afternoon. The total alkalinity ranged from 10 to 240 mg L^{-1} $CaCO_3$, showing mean values of 133.26, 134.17, 131.53 and 133.26 mg $CaCO_3$ L^{-1} for treatments BFT, $BFT_{2.5}$, BFT_5 and BFT_{10} respectively. The lowest value was obtained on the first day of the experiment, and immediately corrected with the use of sodium bicarbonate.

Total ammonia nitrogen (TAN) showed mean values of 1.66, 1.56, 1.36 and 1.5 mg L^{-1} for treatments BFT, $BFT_{2.5}$, BFT_5 and BFT_{10} respectively. The maximum TAN concentration was 4.22 mg L^{-1} in treatment BFT, corresponding to a toxic ammonia concentration of 0.42 mg L^{-1}. The mean concentration of ammonia (Figure 1A) from the 35th day of culture, showed an increase for all treatments, with a peak on day 49.

Nitrite showed no accumulation during the experiment (Figure 1B); but did show an oscillation. The maximum concentration of nitrite nitrogen was 2.85 mg L^{-1}, equivalent to 9.36 mg L^{-1} of nitrite (NO_2). At the start of the culture, high values for nitrate were seen, since the biofloc used in the experiment came from a previous culture, where the system was already mature and balanced. The mean concentrations of this compound did not show the same trend for each treatment (Figure 1C), the maximum concentration being obtained with treatment $BFT_{2.5}$ after 21 days of culture (138 mg L^{-1} NO_3), showing a decrease and oscillation on subsequent days. In the present study, orthophosphate levels ranged from 19 to 858 mg L^{-1}, with mean values of 60.25 and 106.50 for treatments BFT and BFT_{10}, respectively (Figure 1D).

Table 1. Values show the average of four replications ± standard deviation (minimum-maximum) for the physical and chemical variables of water quality in the culture of *O. niloticus* in bioflocs with inoculation of *Chlorella vulgaris* at different densities.

Variables		Treatment			
		BFT	$BFT_{2.5}$	BFT_{5}	BFT_{10}
Temperature	Morning	26.50±0.60	26.50±0.50	26.40±0.50	26.60±0.60
		(24.90-27.80)	(25.10-27.50)	(25.00-27.40)	(25.10-27.90)
	Afternoon	28.20±0.60	28.20±0.60	28.30±0.60	28.30±0.70
		(26.80-29.80)	(26.80-29.70)	(26.80-29.70)	(26.90-30.10)
DO	Morning	6.49±0.43	6.41±0.43	6.53±0.43	6.54±0.45
		(5.20-8.20)	(5.40-8.50)	(5.20-8.30)	(5.30-8.10)
	Afternoon	5.89±0.53	5.86±0.51	5.90±0.51	5.90±0.56
		(3.85-8.00)	(4.40-8.10)	(4.09-7.90)	(4.70-8.20)
pH	Morning	8.23±0.42	8.24±0.42	8.24±0.41	8.25±0.40
		(5.93-8.68)	(5.93-8.68)	(5.94-8.68)	(6.04-8.71)
	Afternoon	8.14±0.31	8.15±0.31	8.14±0.31	8.15±0.31
		(6.07-8.67)	(6.05-8.63)	(6.08-8.65)	(6.14-8.84)
Alkalinity		133.26±42.68	134.17±43.57	131.53±43.98	133.26±45.43
		(10-210)	(10-190)	(10-210)	(10-240)
Ammonia		1.43±1.12	1.40±0.88	1.09±0.88	1.16±1.00
		(0.50-4.22)	(0.31-3.75)	(0.36-2.90)	(0.36-3.70)
Nitrite		0.25±0.61	0.23±0.31	0.25±0.72	0.34±0.74
		(0.03-2.03)	(0.03-1.00)	(0.06-2.85)	(0.07-1.73)
Nitrate		89.00±15.58	88.00±20.24	84.00±18.49	79.00±17.34
		(60-110)	(60-138)	(42-108)	(42-110)
Orthophosphate		60.25±91.84	67.50±129.33	83.50±129.90	106.50±215.63
		(19-396)	(24-530)	(24-506)	(32-858)
SS		16.00±5.90	15.00±4.99	14.50±5.84	17.00±7.05
		(7-34)	(5.5-25)	(2-25)	(7-45)
TSS		276.89±121.84	281.40±101.97	263.98±111.49	261.67±106.85
		(161.36-580.00)	(83.81-568.27)	(98.91-591.26)	(163.95-552.22)

DO - Dissolved oxygen; SS - Settleable solids; TSS - Total suspended solids.

The concentration of sedimentable solids (SS) and total suspended solids (TSS) varied throughout the 63 days of culture, reaching maximum values of 45 mL L^{-1} (Figure 2A) and 591.26 mg L^{-1} (Figure 2B), respectively, and showing no significant difference for inoculation densities of *Chlorella vulgaris* ($P>0.05$).

Zootechnical performance

The mean values for final weight, weight gain, daily weight gain, specific growth rate, survival, feed conversion ratio, final biomass and tilapia productivity after 63 days of culture are shown in Table 2.

Mean final weight ranged from 20.53 to 23.42g for the treatments under study, while daily weight gain presented a mean of 0.3g day^{-1}. The values for specific growth rate were 3.83 to 4.04% day^{-1} for treatments $BFT_{2.5}$ and BFT_{10} and were not influenced by the different inoculation densities of the microalgae *Chlorella vulgaris*. The feed conversion ratio ranged from 1.38 to 1.51, with no significant differences between treatments ($p<0.05$). Survival rates greater than 80% were achieved during this experimental stocking stage.

Hematological analysis

The results of the erythrocyte count, corpuscular volume (hematocrit) and mean corpuscular volume (MCV), and the reference values for variations in blood parameters as per Tavares-Dias (2015) are described in Table 3.

Treatment BFT_{5} showed a higher number of erythrocytes ($4.05x10^{6}$ μL^{-1}), with a significant difference in relation to the other treatments ($p<0.05$). However, no significant differences were found in relation to the hematocrit, the percentages agreeing with the reference values. The highest value for MCV was for treatment BFT_{10}, $131.57x10^{6} fL$.

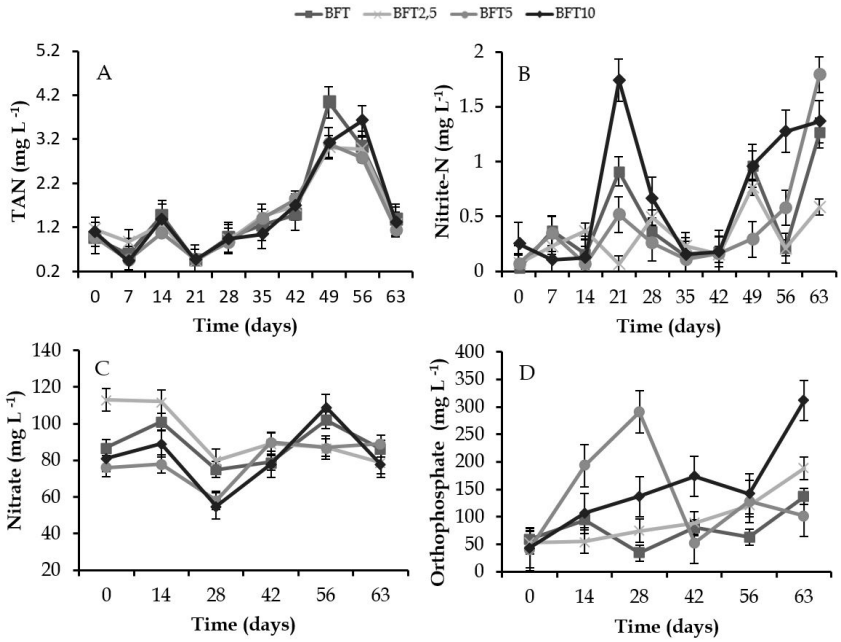

Figure 1. Variation in nitrogen compounds (A- total ammonia nitrogen, B- nitrite nitrogen, C- nitrate, and D- orthophosphate) during the period of *O. niloticus* culture in bioflocs with inoculation of *Chlorella vulgaris* at different densities.

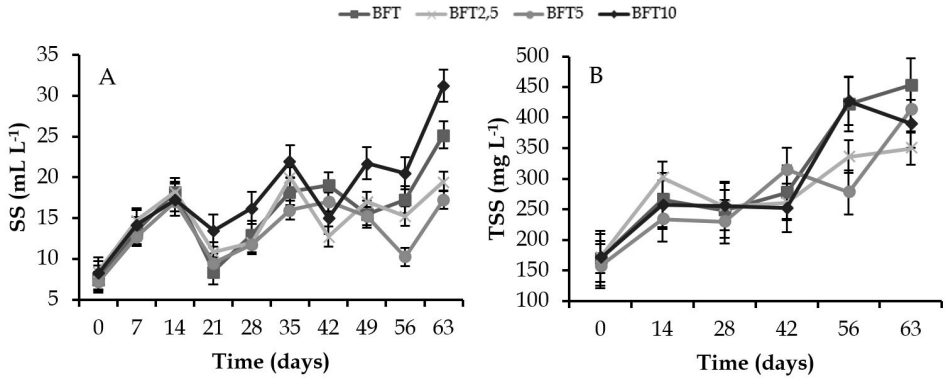

Figure 2. Variation in settleable solids (A) and total suspended solids (B) during the period of *O. niloticus* culture in bioflocs with inoculation of *Chlorella vulgaris* at different densities.

Table 2. Values show the average of four replications ± standard deviation for the variables of zootechnical performance in the culture of *O. niloticus* in bioflocs with inoculation of *Chlorella vulgaris* at different densities.

Variable	Treatment			
	BFT	$BFT_{2.5}$	BFT_{5}	BFT_{10}
Initial weight (g)	1.86±0.08	1.85±0.05	1.88±0.05	1.87±0.06
Final weight (g)	22.23±3.03	20.53±2.97	21.31±1.63	23.42±3.65
Weight gain (g)	20.37±3.02	18.67±2.98	19.12±1.94	21.55±3.61
DWG (g day^{-1})	0.32±0.05	0.30±0.05	0.31±0.03	0.34±0.06
SGR (%day^{-1})	3.96±0.21	3.83±0.24	3.88±0.14	4.04±0.25
Survival (%)	80.00±11.55	82.50±9.57	85.00±17.32	80.00±8.17
FCR	1.51±0.20	1.47±0.11	1.42±0.13	1.38±0.11
Final biomass (g)	179.41±45.50	173.23±28.64	189.63±48.64	186.32±26.13
Productivity (kg m^{-3})	4.49±1.14	4.33±0.72	4.74±1.22	4.66±0.65

SGR - Specific growth rate; DWG - Daily weight gain; FCR - Feed conversion ratio.

Table 3. Values show the average of four replications ± standard deviation for hematological variables in the culture of *O. niloticus* in bioflocs with inoculation of *Chlorella vulgaris* at different densities.

Treatment	Erythrocytes (x10^6 µL^{-1})	Hematocrit (%)	Mean Corpuscular Volume (*f*L)
BFT	3.96±1.37^{ab}	27.00±7.56^{a}	74.49±35.01^{b}
$BFT_{2.5}$	3.69±0.94^{ab}	25.8±6.01^{a}	73.36±21.93^{ab}
BFT_{5}	4.05±0.99^{b}	29.93±7.88^{a}	75.58±15.46^{ab}
BFT_{10}	2.83±1.49^{a}	27.07±9.04^{a}	131.57±120.21^{a}
Reference values*	1.50 – 3.88	21 – 44	74.5 – 160

Different letters on the same line denote a significant statistical difference. *Tavares-Dias (2015).

A low correlation was seen for the variables erythrocytes and mean corpuscular volume, with correlation coefficients (r) of -0.31 and 0.33, respectively. However, a negligible correlation was found (0.04) for the hematocrit percentage.

DISCUSSION

Water quality

The temperature remained within the ideal range of thermal comfort for species growth according to the recommendation of Furuya et al. (2013), which is from 25 to 31 °C. Emerenciano et al. (2017), reported that the minimum concentration of dissolved oxygen for the culture of tilapia in a biofloc system should be ≥ 4mg L^{-1}; the levels of this variable were therefore within the recommended standards throughout the experimental period. The pH presented ideal mean variations for fish development; according to Emerenciano et al. (2017), this variable should be kept between 6.8 and 8.0, and may have favored the growth and maintenance of nitrite-oxidizing bacteria (NOB), which require pH values between 7.2 and 8.2 (Timmons and Ebeling, 2007).

In aquaculture, one of the main objectives in the control of water quality is management of the ammonia, in order to maintain levels at low concentrations (Choo and Caipang, 2015). El-Sherif and El-Feky (2008) evaluated different concentrations of toxic ammonia in relation to the zootechnical performance of *O. niloticus* fingerlings and found a median value for lethal concentration of 7.1 mg L^{-1} N-NH_3, greater than the values found in this experiment.

Nitrite, as well as ammonia, is toxic to aquatic organisms, and its main source is from the oxidation of ammonia (Silva, 2013; Samocha et al., 2017). There was no accumulation of this compound, although this is common under intensive systems, as it is an intermediate compound between the processes of nitrification and denitrification in the nitrogen cycle (Azim and Little, 2008; Kroupova et al., 2005). The maximum concentration obtained was well below 28.1 mg L^{-1} nitrite, which can cause 50% mortality in tilapia fingerlings after 96 hours exposure (Yanbo et al., 2006). Nitrate, a nitrogen compound with a lower toxic potential, is the final product of nitrification (Timmons and Ebeling, 2007), and due to this process, tends to accumulate in intensive culture systems (Kuhn et al., 2010). No nitrite or nitrate accumulation was seen during the experimental period, possibly due to the use of sedimentation tanks.

For alkalinity levels to be maintained between 100 and 150 mg $CaCO_3$ L^{-1} and for there to be no decrease in pH due to non-compensation of the alkalinity, sodium bicarbonate was used as the carbonate source. Reducing alkalinity in the system limits the amount of inorganic carbon available for the bacterial nitrification process, so it is important to maintain alkalinity at levels greater than 100 mg $CaCO_3$ L^{-1} (Samocha et al., 2017).

One characteristic of the system of intensive culture with no exchange of water is the accumulation of the nutrient orthophosphate (Burford et al., 2003; Hopkins et al., 1993). The variation found during the experimental period may be related to the use of sedimentation tanks, agreeing with the findings of Ray et al. (2010) and Schveitzer et al. (2013).

The mean values found in relation to sedimentable solids (SS) agreed with those quoted by Emerenciano et al. (2017), who state that levels should be kept between 5 and 20 mL L^{-1} in the culture of Nile tilapia fingerlings. With the addition of an organic carbon source there is an increase in SS production, mainly composed of heterotrophic and autotrophic bacteria (Luo et al., 2012) that increase the total suspended solids (TSS) by increasing their biomass, in addition to the protein content of the diet and rate of nitrogen excretion of the fish (Monroy-Dosta et al., 2013). According to Emerenciano et al. (2017), the recommended concentration for TSS is less than 500 mg L^{-1}, providing the mean values of the treatments are within recommended levels.

During the second, fifth and seventh week of culture, sedimentation tanks were installed in order to reduce the number of solids in the experimental units, resulting in a reduction in nitrate and orthophosphate (Ray et al., 2010). However, this action may have led to an increase in TAN and NO_2-N concentrations, since according to Ebeling et al. (2006), the use of sedimentation tanks with the aim of removing suspended solids may also remove nitrifying bacteria from the system and consequently impair control of the ammonia.

Zootechnical performance

Miranda-Baeza et al. (2017), when inoculating different densities of the cyanobacteria *Oscillatoria* sp. into a culture of red tilapia fingerlings (*O. mossambicus* x *O. niloticus*) in bioflocs, obtained a

daily gain of 0.14 to 0.2 g day^{-1} during a seven-week experimental period. Those authors started the culture with individuals weighing an average of 14 g, from which they reached a final weight of from 21 to 24 g, values that were below those reached in the present study, demonstrating that cyanobacteria are harmful to the culture. Just as Abduljabbar et al. (2015) found values of 0.1 g day^{-1} when testing the influence of biofloc on the growth of tilapia fingerlings compared to a system with the total and partial exchange of water. Those authors reported that the initial weight of the individuals was between 3.37 and 3.44 g and after 120 days of the experiment achieved an average weight of around 12.16 and 15.65 g, respectively.

The results for specific growth rate were slightly above those found by Zapata et al. (2017), who used 750 juveniles m^{-3} and obtained rates varying between 3.35 and 3.54% day^{-1} when testing different C:N ratios. The results were also greater than those found by Luo et al. (2014), in an experiment conducted in a system of water recirculation (1.90% day^{-1}) and a biofloc system (2.13% day^{-1}), using tilapia with an initial weight of 24 g. Silva et al. (2002), evaluating the zootechnical performance of Nile tilapia fingerlings stocked at different densities (180, 240 and 300 fish m^{-3}) and cultured in an intensive system, obtained 2.19 to 2.91% day^{-1}, lower results than were obtained during the present experimental period.

The feed conversion ratio, which allows the cost-benefit ratio of the feed in an aquaculture activity to be determined (Ogello et al., 2014), was lower than that found by Abdel-Tawwab et al. (2010), who used commercial feed with a protein content of 25, 35 and 45% and obtained 1.81, 1.65 and 1.49 for fingerlings of 0.4 to 0.5g, while for juveniles between 17 and 22g, they obtained 2.22, 1.92 and 1.98, respectively. When the influence of partial water exchange on a culture of tilapia fingerlings (initial weight 2.63 g) was tested in a biofloc system, Abduljabbar et al. (2015) achieved greater values for the feed conversion ratio than those found in this study, of between 1.87 and 1.94, at a temperature of 24 °C.

With survival, Brol et al. (2017), testing different storage densities, obtained 72.9 and 87.5% for 800 and 400 m^{-3} fish, respectively, and Abduljabbar et al. (2015) found rates of 98 and 99% when growing tilapia fingerlings in a biofloc system, one with water renewal and the other exchanging 10% of the water of the culture units; these values were greater than the present values.

Hematological analysis

Treatment BFT_5 presented erythrocyte values above the range considered standard. This increase may be related to a response to the condition of acute stress, since there was a higher concentration of NO_2-N in this treatment on the last day of the culture when the hematological variables were analyzed (Azevedo et al., 2016; Abduljabbar et al., 2015; Long et al., 2015). This compound primarily acts on the blood of individuals, starting with the blood plasma and diffusing into the red blood cells (Cameron, 1971); however, in this treatment, the hematocrit and MCV were within recommended values.

The hematocrit percentage in the treatments under test agreed with that found by Rodrigues-Soares et al. (2018), who obtained values between 27 and 32% when observing the effect of both supplementing and not supplementing with the essential oil of the herb *Lippia alba*.

Values below the parameter recommended by Tavares-Dias (2015) were found for mean corpuscular volume in treatments BFT and $BFT_{2.5}$. It can therefore be seen that, in relation to the variables under analysis, treatment BFT_{10} maintained the normal standards for tilapia cultured in an intensive system, demonstrating the healthy state of the individuals.

CONCLUSION

Weekly inoculation of the microalgae *Chlorella vulgaris* at different densities had no effect on either water quality or the indices of zootechnical performance during the experimental period. However, an improvement could be seen in the MCV index, which indicates the welfare of the animals, in the treatment with *Chlorella vulgaris* inoculated at a density of $10x10^4$ cell mL^{-1}.

From the results, further studies are necessary to evaluate the addition of this microalgae in different concentrations and at varying frequencies, as well as the use of other species, especially those forming colonies, in order to facilitate aggregation on the microbial flakes, and evaluate whether the microalgae has an influence on animal health during transfer between the phases of the culture.

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