BOLETIM DO INSTITUTO DE PESCA
ISSN 1678-2305 online version
Scientific Article

ZOOTECHNICAL PERFORMANCE EVALUATION OF THE USE OF BIOFLOC TECHNOLOGY IN NILE TILAPIA FINGERLING PRODUCTION AT DIFFERENT DENSITIES

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Received: February 20, 2019
Approved: June 30, 2019

ABSTRACT

The effect of producing and culturing Nile tilapia (*Oreochromis niloticus*) fingerlings in biofloc technology (BFT)-based systems was investigated in terms of zootechnical performance, aiming to define the best culturing density for 64 days. A completely randomized design was used, with four replications totaling 24 experimental units. The weight gain of each batch of animals per experimental unit (E.U.) was evaluated at densities of 200, 400, 600, 800, and 1,000 fingerlings m^{-3}. For controls, a Water Recirculation System with 248 fingerlings m^{-3} was used. The fingerlings were fed commercial feed containing 35% protein and brown sugar as a carbohydrate source at a C:N ratio of 20:1. Dissolved oxygen, total ammonia nitrogen, pH, and temperature were monitored daily, and nitrite and alkalinity were monitored weekly. The analysis of weight gain data obtained a linear function $y = - 0.0017X^2 + 3.724X – 87.77$, with $r^2 = 0.864$, coefficient of variation = 14.23%, and correlation coefficient = 0.91. At the end of the cultivation period the system's planktonic community presented high diversity, dominated by rotifers and diatoms. The data of survival, density management demand, and physicochemical parameters variations of the water suggested an optimal density of 800 fish m^{-3}, since this resulted in an average weight gain per E.U. of 1,891.25 ± 151.24 g, with a productive efficiency index of 274.96 that is approximately three times that of the control treatment (87.43). Biofloc technology can be employed in two-phase super-intensive Nile tilapia culture systems, with a stocking density in the rearing stage of 800 fish m^{-3}.

Key words: aquaculture; fish farming; production systems; weight gain.

AVALIAÇÃO DO DESEMPENHO ZOOTÉCNICO USANDO A TECNOLOGIA DE BIOFLOCOS NA PRODUÇÃO DE ALEVINOS DE TILÁPIA DO NILO EM DIFERENTES DENSIDADES

RESUMO

O efeito da produção e cultivo de alevinos de tilápia do Nilo (*Oreochromis niloticus*) em sistema baseado na tecnologia de bioflocos (BFT) foi investigado no que se refere ao desempenho zootécnico, visando definir a melhor densidade de cultivo por 64 dias. Foi usado um delineamento inteiramente casualizado, com quatro repetições, totalizando 24 unidades experimentais. O ganho de peso dos animais por unidade experimental (U.E.) foi avaliado nas densidades de 200, 400, 600, 800 e 1000 alevinos m^{-3}. Como controle foi usado um Sistema de Recirculação de Água com 248 alevinos m^{-3}. Os alevinos foram alimentados com ração comercial contendo 35% de proteína e açúcar mascavo como fonte de carboidratos, em uma proporção C:N de 20:1. Diariamente foram monitorados os parâmetros oxigênio dissolvido, amônia total, pH e temperatura, e semanalmente nitrito, alcalinidade, além das biometrias. A análise dos dados de ganho de peso obteve uma função linear $Y = - 0,0017X^2 + 3,724X - 87,77$, com $r^2 = 0,864$, CV = 14,23% e correlação de 0,91. Ao final do cultivo a comunidade planctônica no sistema apresentou grande variedade, predominando rotíferos e diatomáceas. A junção dos dados de sobrevivência, demanda de manejo por densidade de cultivo e variação dos parâmetros físico-químicos da água sugeriram uma densidade ótima de 800 peixes m^{-3}, resultando em ganho de peso médio de 1.891,25 ± 151,24 U. E., com o índice de eficiência produtiva de 274,96, aproximadamente três vezes o valor encontrado no tratamento controle (87,43). A tecnologia de bioflocos pode ser empregada em sistemas super-intensivos bifásico no cultivo de tilápia do Nilo, com uma densidade de estocagem de 800 peixes m^{-3}.

Palavras-chave: aquicultura; piscicultura; sistemas de produção; ganho de peso.

INTRODUCTION

In recente decades, the demand for fish has increased worldwide, mainly because of the increase in human population and preoccupation with the consumption of healthy foods (Brabo et al., 2016). This demand justifies the investment that has been made in aquaculture, considering that fish production through fishing has stagnated both in Brasil and worldwide since the mid-90s. The average production of Brazilian extractive fisheries, which was at 243 thousand tonnes between 2005 and 2014, decreased to 225 thousand tonnes in 2014; a similar production volume was estimated for 2018 (FAO, 2018).

In Brazil, the production of aquaculture in 2016 was estimated at 600 thousand tonnes (FAO, 2018), Tilapia (*Oreochromis* sp.) was the most produced aquatic organism, accounting 50.64% of the Brazilian continental fish farming in 2016 (IBGE, 2017). Worldwide, Nile tilapia (*Oreochromis niloticus*) represents approximately 58% of farmed fish (FAO, 2018). Tilapia produces white meat and has an excellent bone structure for filleting, good yield, and excellent market value (Kubitza, 2011; SEBRAE, 2015).

Traditional aquaculture has a high water consumption activity (FAO, 2016), making it unsustainable in most of the Northeast region of Brazil, which is historically known for its severe droughts. However, through the application of new management technologies, it is possible to farm aquatic organisms in regions of low rainfall and scarce water availability. In this context, a culturing technique using bioflocs presents a feasible alternative, since it allows for fish farming with minimum water use and less dependence on exogenous feeding (Das and Mandal, 2018).

Fish farming and culturing occurs in four cropping systems: super-intensive, intensive, semi-intensive, and extensive (Lima, 2013). Extensive and semi-intensive systems require less applied technology and lower energy expenditure, but they demand a great quantity of water per kilogram of fish produced, and is thus restricted to places where there is high water availability in terms of both quantity and quality. In intensive and super-intensive systems, the application of more refined production techniques, with the use of water filtration and recirculation, requires the use of electric energy for the operation of the filters, but with a reduced consumption of water. Another factor to be considered is feed consumption, which in intensive and super-intensive systems is the only source of food for the animals in cultivation (Kubitza, 2011).

The technique based on bioflocs technology (BFT) is characterized as super-intensive; it is being based microbial community comprised by aggregates of bacteria, microalgae, protozoan and other invertebrates that boost natural productivity, water quality and nutrient cycling (Pinho et al., 2017). Bioflocs assimilate ammonia nitrogen, offering a rich natural feed for planktonic organisms (Avnimelech, 1999; Schneider et al., 2006; Ekasari et al., 2010). The basic principle of this system is to control the carbon and nitrogen (C:N) ratio in the water at predefined concentrations, in order to guarantee the survival of heterotrophic microorganisms and thus maintain the production system (Ren et al., 2018).

The main control factors that must be regulated in aquaculture are the alkalinity, concentration of suspended solids, aeration, and C:N ratio in the culture water (Ebeling et al., 2006; Avnimelech and Kochba, 2009). Biofloc cultivation technology, replacing traditional aquaculture production models, offers an environmental improvement by dispensing with water renewal and can also be used to test alternative diets that contribute to the reduction of environmental effects due to the decreased need for nutrient inputs (Emerenciano et al., 2012; Poli et al., 2018; Ren et al., 2018). In order to choose the carbon source, one must take into account its availability and ease of acquisition, as well as its carbohydrate content (Emerenciano et al., 2017). Among the sources tested are glucose (Ekasari et al., 2010), starch (Crab et al., 2007), wheat bran, sugar (Poli et al., 2018), molasses (Lima et al., 2018), and sugarcane bagasse (Hargreaves, 1998). Among the sugars obtained directly from concentrated sugarcane juice (*Saccharum officinarum*), brown sugar is a carbohydrate that does not receive chemical treatment during production, is rich in vitamins and minerals, and is commercially available at sucrose contents that vary between 90% and 99.8% (Machado et al., 2012). In this study, brown sugar was used for its ease of acquisition in the local market and for having a known centesimal composition, which dynamizes the experimental stage.

The utilization of biofloc production systems is increasing globally, including in Brazil. Biofloc studies have been carried out for several species of fish, including African catfish (*Clarias gariepinus*) (Dauda et al., 2018), jundiá (*Rhamdia quelen*) (Poli et al., 2015), carp (*Cyprinus carpio*) (Najdegerami et al., 2016), and piracanjuba (*Brycon orbignyanus*) (Sgnaulin et al., 2017). The BFT applied to tilapia culture has been studied by several researchers, both in the initial stages of cultivation (Lima et al., 2015; Brol et al., 2017) and the fattening phase (Azim and Little, 2008; Kubitza, 2011; Mansour and Esteban, 2017).

As bioflocs technology is a cropping system with little water exchange, its application in fish farming systems in northeastern Brazil is a perfectly applicable alternative, since it would allow small and large producers to produce fish in places with low water availability. On the other hand, the BFT system would enable producers to produce large quantities of fry in a small area of cultivation. There would be an increase in both income for farmers and supply in the market of quality fingerlings capable of maintaining producing for the region, in one-, two-, or three-phase systems. Based on this, the main objective of this study was to define the best culture density by analyzing productive efficacy of bioflocs technology in a tilapia nursery based on zootechnical performance data.

MATERIAL AND METHODS

This work was approved by the Animal Use Ethics Committee (AUEC) of the Universidade Federal do Recôncavo da Bahia (UFRB), under certificate number 23007.027487/2017-60, as established by the National Council for the Control of Animal Experimentation. The experiment was carried out at the Nucleus of Studies in Fisheries and Aquaculture (NEPA), UFRB. Chitralada variety Nile tilapia were donated by Aquavale fishery, Ituberá, Southern Bahia Lowlands.

The tests were carried out in 250 L tanks with a useful volume of 150 L each, for a duration of 64 days. The production of tilapia using BFT was tested for five fish densities: 200 (T1), 400 (T2), 600 (T3), 800 (T4), and 1,000 (T5) fingerlings m^{-3}. Each treatment was tested in four replicates according to the method of Champely (2015), assuming a Cohen F^2 effect size, estimated at 0.04, and based on the works of Ekasari et al. (2010), Santos et al. (2013), and Martins et al. (2017). In all, 2,048 fingerlings were used, divided between the five treatments plus the control, totaling 24 tanks (PVC) each with a volume of 250 L.

The control treatment (CT) served as a reference for the zootechnical performance, since it was structured in a system with water recirculation (SWR). The CT consisted of four tanks of 250 L each with a density of 248 specimens m^{-3} based on the calculation of Cochran (1963). The CT experimental units received a shared filter with a 100 L of useful volume, with both mechanical (20 kg of inert stones and 10 mm granulometry) and biological filtration (23 L of bioball). This type of media offered an area to volume ratio of 1,700 m^2 m^{-3}, coupled to a discharge pressure pump with a capacity of 1,300 L h^{-1}. Radioactive filtering was also used via the application of ultraviolet light (UV), with a power of 36 W.

For the establishment of a microbiota of heterotrophic nature constituent of the biofloc system (inoculum), a 100 L tank filled with 80 L of water was initially used, adjusted to an alkalinity of 120 ppm, pH at 7.8, salinity at 5 ppt, forced aeration by means of an air compressor with power of 3.5 W and flow rate of 6 L min^{-1}, connected by means of ¼” silicone hoses to two diffusers, cylindrical porous stone type, with dimensions of 52 mm × 22 mm, built in non-toxic mineral. Following the methodology of Avnimelech (1999), a C:N ratio of 20:1 was established. The nitrogen source used to stabilize the biofloc was urea (46% N), and the carbohydrate source was brown sugar (90% CHO), which was previously diluted and added in several steps daily to the water, until 5 mL L^{-1} of decantable suspended solids was observed and the stabilization of the total ammonia nitrogen stabilized at values close to zero, which occurred after 64 days.

At this stage, concentrations of dissolved oxygen and alkalinity were monitored and maintained, the latter determined by volumetric titration using 0.01 M sulfuric acid (H_2SO_4) as a titrant and bromothymol blue ($C_{27}H_{28}Br_2O_5S$) as an indicator, assuming, in this context, pH, and that the alkalinity of the carbonate was equivalent to the total alkalinity, following the methodology of Macêdo (2005), where the alkalinity levels were maintained close to 100 ppm. In order to maintain alkalinity levels, the sodium bicarbonate ($NaHCO_3$) was diluted beforehand.

The inoculation of the experimental units occurred in successive stages, and was carried out in the proportion of one part of water with bioflocs to five parts of water in 250 L tanks at an initial volume of 80 L. The initial physical and chemical parameters were an alkalinity of 120 ppm, pH of 7.8, salinity of 5 ppt, and constant aeration, and at the end of the spreading, all 20 experimental units were homogenized.

All tanks were aerated using two turbine blowers, the first with a power of $^1/_6$ CV and flow of 11,400 L h^{-1} and the second with a power of ½ CV and flow of 37,200 L h^{-1}, both connected to porous stone diffusers. The lower power compressor was connected to a backup power system, based on an energy-saving automatic keyed device consisting of a deep discharge stationary type battery with a nominal 12 V output voltage and 115 A h^{-1} current, which was connected to a power inverter with a maximum rated current of 600 VA and a selectable output of 110-220 V. The system also had an intelligent charger, installed with the objective of automatically recharging the battery whenever necessary. This setup ensured the safe aeration and movement of the water mass in all tanks in the case of power loss.

Reception and acclimatization of fish

The fingerlings were acclimatized in eight 250 L tanks containing clean water with a salinity of 5 ppt and a pH adjusted to 7.5. The fish remained in quarantine for seven days. On the eighth day, the fish were transferred to the experimental units and the biometrics were collected for 10% of the fingerlings randomly.

During the quarantine, the total ammonia nitrogen reached a value of up to 2 mg L^{-1}, being controlled by the 30% exhange of the total effective volume of the system. The initial mortality was 2%, and the carcasses of the animals were removed from the tanks and composted. The initial batch exhibited great homogeneity of weight and size, presenting an initial mean weight of 1.0 ± 0.072 g and a mean length of 3.0 ± 0.3 cm. At the end of the acclimation period, the fingerlings were transferred to the experimental units. Mortality in the biofloc systems was lower than 0.2%, with no mortality in the control system. In order to reduce the stress of the animals and not interfere with the weight gain data, we opted for the suspension of feeding on the days when the biometrics were collected.

Feeding of animals and maintenance of bioflocs

All fingerlings were fed a commercial feed containing 35% crude protein, 5.0% lipids, and 7.0% fibrous matter, with a maximum moisture of 13%. The amount supplied was calculated according to the stage and biomass present in each treatment. The daily food quantity was divided and provided in three daily portions: morning, midday, and afternoon. The ration at each stage was weighed using a semi-analytical balance (brand BEL, model S423) to the nearest 0.001 g. The amount of total feed given in each tank was recorded for later determination of the apparent feed conversion. For the maintenance of the bioflocs, a C:N ratio of 20:1 was used. The source of carbon was brown sugar. The sugar was previously weighed and diluted in chlorine-free water and applied to the tanks in a split-order manner at intermediate feeding times.

Physicochemical parameters of water quality

Water quality was estimated based on the following physical and chemical variables: temperature (ºC), salinity (ppt), dissolved oxygen (mg L^{-1}), total ammonia nitrogen ($N\text{-}NH_3$ + $N\text{-}NH_4$), and pH. The listed parameters were measured twice a day at 0800 and 1700 h, using two multiparameter probes (YSI model Professional Plus [Pro Plus] and HANNA pHep). Water samples were collected from each once a week for the analysis of the nitrite nitrogen (N-NO_2) and alkalinity ($CaCO_3$). The total ammonia nitrogen tests were performed using the phenol-hypochlorite method (Weatherburn, 1967). The nitrite nitrogen tests were performed on the basis of the sulfanilic acid/alpha-naphthylamine reaction yielding a rosy solution that was analyzed in a spectrophotometer at 540 nm, following the method described in normative instruction number 20 of July 21, 1999 (Brasil, 1999).

For the suspended solids analysis, 1 L water samples per experimental unit were collected in beakers and allowed to settle for 40 min. The concentrations of sedimented suspended solids were maintained at values close to and less than 20 mL L^{-1} (Avnimelech, 1999).

Biometrics

The weight (g) and total length (mm) of 20% of the individuals, randomly selected in each of the tanks, were sampled weekly. In order to increase the biometric precision, the fish were weighed and measured individually. After obtaining the average weight, this was extrapolated to the lot, thus defining the weight of each lot of fish allocated in each experimental unit. The average calculated weight was used as the estimated average for each tank, and was then used to adjust the total feed biomass and amount of added carbohydrates.

The length measurements were performed with a stainless steel pachometer (Vernier Caliper brand) of 20 divisions, to the nearest 0.05 mm. At the end of the experiment, the remaining fish were counted in each treatment to calculate the survival rate (SR).

Evaluation of zootechnical performance

The parameters used for the evaluation of the zootechnical performance are presented in Table 1.

Qualitative survey of the planktonic microbiota present in the system using BFT

The qualitative analysis of the planktonic microbiota was performed on the first and last weeks of the experimental by using two 150 mL water samples from each tank, including the control group, totaling 48 samples. For the microorganism identification, the samples were fixed and observed under an optical and stereoscopic microscope using a Sedgewick-Rafter chamber. The zooplankton groups were identified at the lowest possible taxonomic level using taxonomic keys, comparisons with illustrative photos, and a specialized bibliography (Elmoor-Loureiro, 1997; Reid, 1999).

Table 1. Indexes of zootechnical performance of Nile tilápia fingerlings grown in a biofloc system and their formulas.

ÍNDEXES	FORMULAS
Weight gain per E.U	WGEU = (G_{last} – $G_{initial}$)
Daily weight gain	DWG = (G_{last} – $G_{initial}$)/days
Survival rate	SR = ($Population_{last}$ /$Population_{initial}$) x 100
Feed conversion rate	FCR = Food provided (g)/WG(g)
Producvitivity	P = Biomass total (kg)/Volume (m^3)
Productivity efficiency index	PEI = [(DWG x SR)/FCR] x 100

The experiment was delineated in a completely randomized design, with five treatments at different densities (BFT) and one control treatment (SWR), where the response variable analyzed was weight gain. For the weight gain data, we performed an analysis of variance (ANOVA) and a posterior regression analysis, in order to determine the best culture density. Tukey's test was also applied for post hoc analysis in order to improve the interpretation of the data that were processed using the statistical computer program R. For the verification of normality and homoscedasticity, the tests of Shapiro-Wilk and Bartlett were performed, respectively, which confirmed the validity of the statistical analysis.

RESULTS

During the cultivation (fifth experimental week), a feeding pause was required for three consecutive days due to an energy decrease, which consequently caused an accumulation of total ammonia nitrogen and decreased the concentrations of dissolved oxygen in the system. At this time, the total ammonia nitrogen concentration reached 6.5 mg L^{-1} in 95% of the tanks in the biofloc system, while the oxygen level was at 0.74 mg L^{-1} in tank 20 (T5). As soon as the levels were restored, carbohydrate was added to favor the growth of the heterotrophic microbiota and the subsequent assimilation of the total ammonia nitrogen was observed 72 h after the occurrence. For 24 h, nitrite nitrogen concentrations of up to 28 mg L^{-1} were recorded. At that time, 10% mortalities were recorded in tank 09 (T3), 0.33% in tank 02 (T3), and 0.4% in tank 13 (T4). The partial and unintentional pause in the aeration system lasted for a period of 4 h (fifth experimental week). The variations in mean total ammonia nitrogen and nitrite nitrogen concentrations per treatment are shown in Figure 1A and 1B.

Interruptions to the feeding schedule occurred in another three instances, on the 42nd, 49th, and 56th days of the experimental period, again due to energy interruption. In those instances, there was an increase in the ammonia concentration in 12 tanks, which presented mean values of 3.5 mg L^{-1}. The lowest concentration of oxygen occurred in tank 19 (T5), reaching 2.74 mg L^{-1} (Figure 1C and 1D), but with no mortality. Once again, corrective carbon addition was used, restoring the concentrations of total ammonia nitrogen to less than 1.0 mg L^{-1} in less than 12 h.

During the experimental stage, dissolved oxygen presented different concentrations in the daily measurements performed at 08:00 and 17:00 h (Figures 1C and 1D). In relation to temperature, this varied uniformly and progressively throughout the day for all tanks. The mean temperature values during the experimental period were 26.7 ± 0.71 °C at 08:00 h, 28.6 ± 0.57 °C at 12:00 h, and 31.9 ± 0.89 at 17:00 h.

For the pH, a gradual decrease in the higher density treatments was observed; however, the values remained within a minimum of 7.2. Figure 1E shows the average behavior of the pH levels during the experimental stages, while Figure 1F shows the behavior of the carbonate concentrations, including the alkalinity corrections performed on the 28th and 49th experimental days.

Growth of the microbiota and removal of the sedimentable solids

Each treatment obtained a distinct production of bioflocs, and the higher densities presented higher yields, reflecting the supply of higher amounts of nitrogen and carbohydrate via feed and sugar. As the maximum concentration measured was 20 mL L^{-1} suspended solids in the system, a decanter was used whenever the concentration of solids exceeded that limit. Therefore, the settling frequency varied directly proportional to the stocked density, and indicated the amount of handling required for each treatment (Table 2).

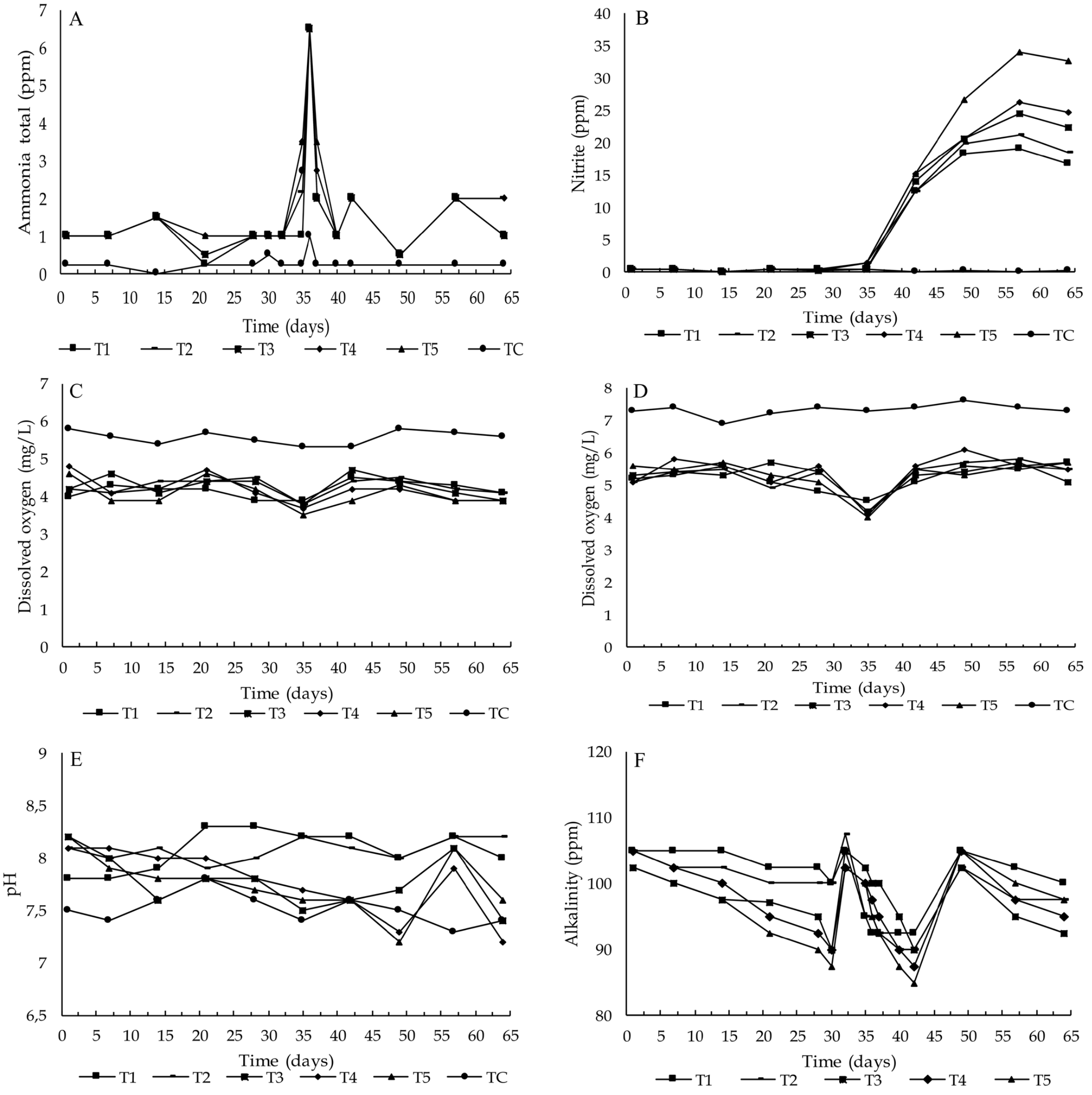

Figure 1. Variation in the mean concentration of total ammonia nitrogen (A), nitrite nitrogen (B), dissolved oxygen at 8 h (C), dissolved oxygen at 17 h (D), pH (E), and alkalinity (F) in tilapia fingerlings cultures of different densities in a BFT - based system (T1 to T5) and control (SWR, TC)." BFT = biofloc technology. SWR = system water recirculation. TC = treatment control.

Table 2. Average frequency of settling chamber by treatment for the cultures of tilapia fingerlings in a biofloc system during a 64-day period.

Treatments	T1	T2	T3	T4	T5
settling chamber	4.0 ± 1.4 c	5.0 ± 1.4 c	7.0 ± 0.81b c	8.5 ± 1.29 b	7.42 ± 0.16 a

The values followed by the same lowercase letter not differ statistically, according to Tukey's test ($p < 0.05$). T1-T5 = treatments.

Qualitative survey of the planktonic community in biofloc cultures

The microbiota identified in the biofloc systems, *Chroococcus* sp., *Microspora* spp., *Ulothrix* sp., *Ostracoda* sp., *Ciliado* sp., *Chroococcus* sp., *Ciliado* sp., *Phormidium* sp., *Lecane* sp., diatoms, *Oscillatoria* sp., and *Lepadella* sp., exhibited a high similarity between the microbiota of each treatment.

Zootechnical performance

The biometric data, food consumption, final biomass, SR, and productive efficiency index, with the respective coefficients of variation, are listed in Table 3.

Regarding weight gain per experimental unit, this was the response variable reported for the statistical analysis to determine the best culture density. The weight gain data per treatment are shown in Figure 2A and 2B.

There was a 91.20% correlation between stocking density and weight gain, reaching a second-degree linear regression function, ($Y = -0.0017X2 + 3.724X - 87.77$), with $r^2 = 0.864$ and a CV of 14.23%. Through the critical point of the regression function, the prospective value of 1,057 fish m^{-3} was obtained as the optimal density, with an estimated weight gain of 1,881 g. The observed range did not cover the prospected density, that being below (200 to 1000 fish m^{-3}). However, the weight gain estimated through the function (1.881 g) is within the range observed for the weight gain data obtained in the T4 and T5 treatments. Additionally, the Tukey test performed using the data of weight gain data in all treatments showed statistical equality between treatments T3, T4, and T5, and T1, T2, and CT (Table 3).

DISCUSSION

The eventual interruptions of the energy showed that systems using BFT, with a safety option that guarantees 25% of the aeration in low-volume tanks with salinities of 5 ppt, is resistant to the momentary decrease in the dissolved oxygen concentrations. These results are in agreement with the findings of other studies, including those of Avnimelech (1999), Crab et al. (2007), and Emerenciano et al. (2012). However, it is to be emphasized that the low volume of water in the experimental units allowed the rapid recovery of oxygen levels to the ideal range. It is also worth emphasizing the tolerance of tilapia to low oxygen concentrations (Baldisseroto, 2013).

Table 3. Zootechnical performance during the cultivation of tilapia fingerlings in a biofloc system treatment (T1 - T5) and control treatment (CT) at different densities for 64 days.

TRAT	WGEU (g)	DWG (g)	FCR	SR (%)	P (kg m^{-3})	PEI (%)
T1	651.75 ± 76.57b	10.18 ± 1.20b	0.94 ± 0.05a	90.83	4.35 ± 0.51b	98.32
T2	989.00 ± 127.93b	15.45 ± 2.00b	1.13 ± 0.13a	88.33	6.59 ± 0.85b	125.77
T3	1517.50 ± 143.90a	28.43 ± 5.88a	0.93 ± 0.08a	89.26	12.13 ± 1.49a	217.09
T4	1891.25 ± 151.24a	29.55 ± 2.36a	0.95 ± 0.05a	87.5	12.61 ± 1.01a	274.96
T5	1811.5 ± 284.02a	28.3 ± 4.44a	1.15 ± 0.1a	86.33	12.08 ± 1.89a	219.19
CT	731.08 ± 37.31b	11.42 ± 0.58b	1.01 ± 0.02a	98.79	4.87 ± 0.24b	87.43
CV(%)	14.23	-	8.55	15.84	13.79	-

The values followed by the same lowercase letter in each column not differ statistically, according to Tukey's test ($p < 0.05$).. TRAT = Treatment; WGEU = Weight gain per experimental unit; DWG = Daily weight gain; FCR = Feed conversion rate; SR = Survival rate; P = Productivity; PEI = Productivity efficiency index; CV = Coefficient of variation.

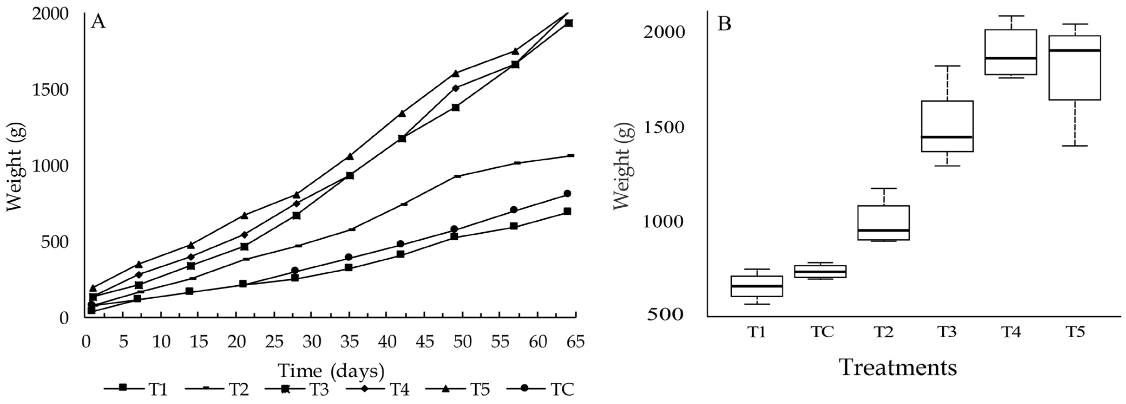

Figure 2. Weight gain per experimental unit (days) (A) and weight gain distribution (B) during the cultivation of tilapia fingerlings in a biofloc system (BFT) and control treatment (SWR) at different densities, for 64 days. BFT = biofloc technology. SWR = system water recirculation. TC = treatment control.

The maximum concentration of ammonia (6.5 mg L^{-1}) was below the 48-h LC_{50} lethal concentration found by Lima et al. (2015) for Nile tilapia fingerlings (7.1 mg L^{-1} of $N\text{-}NH_3$). The low mortality of the fingerlings could be attributed to the high level of acclimation exhibited by tilapia when exposed to low concentrations of dissolved oxygen and high concentrations of nitrogen compounds (Baldisseroto, 2013; Lima, 2013). Another factor that influenced fry survival was the present salt content (5 ppt) in the experimental tanks, since the Cl-ion decreases the nitrite toxicity in aquatic environments (Yanbo et al., 2006; Baldisseroto, 2013).

A positive effect was noted for the addition of a carbohydrate source to a system dominated by predominantly heterotrophic microbiota, favoring the assimilation of the ammoniacal nitrogen from the excretions and feed leftovers; thus, low concentrations were ensured. This decrease in total ammonia by the addition of carbohydrate was also observed in the studies of Avnimelech (1999), Hargreaves (2006), Ray and Lotz (2014), Lima et al. (2018), Ren et al. (2018), and Poli et al. (2018). The effectiveness of BFT was confirmed by observing the average ammoniacal nitrogen concentrations, which presented values within the range tolerated by tilapia, i.e., 1.0 ± 0.5 mg L^{-1} at a C:N ratio of 20:1.

With regard to the dissolved oxygen, it is verified that the average concentrations varied throughout the day, and were higher in the diurnal period. During the day, the concentration of oxygen increases due to photosynthesis, and decreases at night because there is no light. In the nocturnal period, the biological respiration of the BFT system when consuming the excess oxygen at the end of the afternoon causes deficits of oxygen during the dawn and the first hours of the morning (Kubitza, 2017). In the BFT system, the heterotrophic microbiota is responsible for about 60% of the total oxygen consumption (Emerenciano et al., 2017).

Constant temperature was expected once the experimental units were housed in a greenhouse, demonstrating the advantage of cultivating tropical species under such conditions. On rare occasions, temperatures dropped under 26 °C in the morning, which is below the optimum temperature for tilapia (Baldisseroto, 2013; Lima, 2013), but quickly recovered during the course of the morning to reach values within the range of comfort for the species. It is noteworthy that even at these low temperatures no decrease in feeding activity was observed.

pH stability was attributed to the buffer effect described by Martins et al. (2017), where the alkalinity levels were maintained close to 100 ppm by the addition of sodium bicarbonate ($NaHCO_3$). In general, the observed parameters confirmed that BTF adequately fulfills the physico-chemical requirements demanded by Nile tilapia and can be suggested for its culture.

The observation of the qualitatively similar plankton microbiota in all treatments was expected, since the total volume of water from all tanks was homogenized prior to settlement. The variety of microorganism species found in the biofloc tanks resembles those found by Monroy-Dosta et al. (2013). Such variety had a positive influence on the performance of Nile tilapia fingerlings, as it offered a good food supplement generated by bioflocs from the assimilation of the nitrogen compounds present in the culture.

Despite the feeding interruptions at certain intervals of the experiment, the zootechnical performance of the fingerlings was considered optimal, having a feed conversion value of 0.95 for treatment T4 (800 fish m^{-3}), and an SR of 87.5%. This result was superior to that of recent studies, such as that of Brol et al. (2017), who cultured tilapia fingerlings using BFT and obtained a feed conversion rate (FCR) of between 1.21 and 1.29 and SR of between 72% and 87%; and Schwarz et al. (2016), who tested different tilapia fingerling densities (mean initial weight of 4.68 g) in five treatments (n = 540) for 60 days, with an SWR and 35% crude protein feed, and obtained an FCR of between 1.56 and 1.78 and an SR of between 92% and 94%.

A significant challenge to the studies on biofloc systems is determining the best feeding rate; in some systems, leftover feed was observed in the tanks. This suggests the need for more data on the exogenous feeding rate of animals grown in biofloc systems, which has the potential to greatly reduce production cost; in our study, no feed leftovers were observed in the control treatments (SWR), indicating the feeding rate was in agreement with the total consumption.

The SR observed among the difderent treatments, including controls, did not show a significant difference ($p < 0.05$). However, the observed mean SR (88.45%) was lower in all treatments using BFT compared to those using SWR (98.79%). We stress that the observed differences should not only be attributed to the use of two different methods for water treatment, but is also a product of the different densities between the treatments and the controls, which was the aim of the experimental design.

The productive efficiency index (PEI) is used in the analysis and monitoring of the zootechnical performance of commercially produced animals, mainly poultry and swine. This index is calculated on the basis of feed conversion, weight gain, and survival data, and signifies the level of efficiency in the production of a particular animal. In the present study, the highest PEI occurred in the T4 treatment (800 fish m^{-3}), showing that at this density, there was an excellent FCR (0.95) associated with low mortality (12.5%), in addition to a good weight gain per experimental unit (1.89 g). Thus, it is suggested to apply this index in fish cultures, both in the academic and productive sectors in order to subsidize sustainable aquaculture, and also aim to establish public-private partnerships that can use this index as a reference.

Several studies have shown the suppression of territorial behavior in tilapia at high densities, which further supports the practice of high-density culturing (Lambert and Dutil, 2001; Moro et al., 2013; Ren et al., 2018).

Regardless of the densities tested, it was observed that the maintenance of a heterotrophic microbiota reduces the concentration of toxic nitrogenous compounds, concurrently providing a sustainable population of microorganisms that function as a food source, enhancing the zootechnical performance of the culture.

CONCLUSION

The biofloc system is able to assimilate the total ammonia produced by the cultivation of tilapia fingerlings at a density of up to 1,000 fish m^{-3}, offering an aquatic environment rich in microbial protein that maximizes the endogenous supply of food to the population in the culture.

According to our findings, a density of 800 fish m^{-3} is optimal for the cultivation of tilapia fingerlings with an initial weight of 1.0 g in a biofloc system for 64 days. At this density, the mean weight gain per experimental unit was 258.68% higher than that per experimental control unit in SWR under a density of 248 fish m^{-3}. Our results demonstrate that the biofloc system improves the zootechnical performance of tilapia at high densities in the initial stage of cultivation.

We also believe that two- or three-phase tilapia cultivation is possible in the BFT system, but further studies are required to establish the best density in the rearing and fattening stages, as well as the ideal duration for each phase. It is also worth noting that in addition to the high productivity achieved in this system, the main advantages of tilapia raising in BFT in the northeast region of Brazil are the water economy and the decreased need for exogenous feeding, characteristics that offer the possibility of solid and sustainable aquaculture production.

ACKNOWLEDGEMENTS

The authors are grateful financial support in part of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001 and Profa. Dr. Carla Fernandes Macedo for the chemical analysis.

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