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

TILAPIA CULTIVATED IN A LOW-SALINITY BIOFLOC SYSTEM SUPPLEMENTED WITH *Chlorella vulgaris* AND DIFFERENTS MOLASSES APPLICATION RATES

Priscilla Celes Maciel de Lima[1]
Luis Otavio Brito da Silva[1]
Jéssika de Lima Abreu[1]
Suzianny Maria Bezerra Cabral da Silva[2]
William Severi[3]
Alfredo Olivera Gálvez[1]

[1]Universidade Federal Rural de Pernambuco – UFRPE, Laboratório de Maricultura Sustentável, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, CEP 52171-900, Recife, PE, Brasil. E-mail: pri.c.maciel@hotmail.com (corresponding author).

[2]Universidade Federal Rural de Pernambuco – UFRPE, Laboratório de Sanidade de Animais Aquáticos, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, CEP 52171-900, Recife, PE, Brasil.

[3]Universidade Federal Rural de Pernambuco – UFRPE, Laboratório de Limnologia, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, CEP 52171-900, Recife, PE, Brasil.

Received: February 19, 2019
Approved: June 30, 2019

ABSTRACT

The aim of this study was to evaluate the effect of supplementation with *Chlorella vulgaris* and molasses application rates on water quality, zootechnical performance, proximate composition and health status of Nile tilapia (*Oreochromis niloticus*) fingerlings cultivated in low-salinity (10 g L^{-1}) biofloc systems. Four treatments were tested in a factorial design (supplemented with microalgae and molasses application rates): BFT-C30 (Biofloc supplemented with *C. vulgaris* and molasses application rates of 30% of the total daily feed); BFT-30 (Biofloc with molasses application rates of 30% of the total daily feed); BFT-C50 (Biofloc supplemented with *C. vulgaris* and molasses application rates of 50% of the total daily feed) and BFT-50 (Biofloc with molasses application rates of 50% of the total daily feed), for 70 days. Fingerlings of *O. niloticus* (initial mean weight of 3.15 ± 0.5 g) were stocked at a density of 680 fish m^{-3} in experimental units (50L), where 50% of this volume was biofloc previously matured. Throughout the experiment, they were supplemented with *C. vulgaris* every five days at the concentration of $5x10^4$ cells mL^{-1}. A significant interaction between supplementation with *C. vulgaris* and molasses application rates for final weight and length, survival, feed conversion ratio, specific growth rate, water consumption, protein efficiency ratio, sedimentation time, planktonic community and hematological indices were observed. The results indicated that the high molasses application rates (50%) in the biofloc system affects the zootechnical performance, water consumption, sedimentation time and the hematological indices of the Nile Tilapia fingerlings, hampering their development. Therefore, molasses application rates of 30% of the total daily feed for the tilapia fingerlings culture in low-salinity biofloc system is recommended.

Key words: microalgae; carbohydrate; proximate composition; zootechnical performance; hematological indices.

CULTIVO DE TILÁPIA EM BIOFLOCO EM BAIXA SALINIDADE SUPLEMENTADO COM *Chlorella vulgaris* E DIFERENTES TAXAS DE APLICAÇÃO DE MELAÇO

RESUMO

O objetivo deste trabalho foi avaliar o efeito da suplementação com *Chlorella vulgaris* e taxas de aplicação de melaço na qualidade da água, desempenho zootécnico, composição centesimal e saúde de alevinos de tilápia do Nilo (*Oreochromis niloticus*) cultivados em sistemas de biofloco com baixa salinidade (10 g L^{-1}). Quatro tratamentos foram testados em um delineamento fatorial (suplementado com microalgas e taxas de aplicação de melaço): BFT-C30 (Biofloco suplementado com *C. vulgaris* e aplicação de melaço de 30% da alimentação diária); BFT-30 (Biofloco com aplicação de melaço de 30% da alimentação diária); BFT-C50 (Biofloco suplementado com *C. vulgaris* e aplicação de melaço de 50% da alimentação diária) e BFT-50 (Biofloco com aplicação de melaço de 50% da alimentação diária), por 70 dias. Alevinos de *O. niloticus* (peso médio inicial de 3,15 ± 0,5 g) foram estocados na densidade de 680 peixes m^{-3} nas unidades experimentais (50L), onde 50% deste volume foi de biofloco previamente maturado. Durante todo o experimento, as inoculações com *C. vulgaris* foram a cada cinco dias na concentração de $5x10^4$ células mL^{-1}. Foi observada interação significativa entre a suplementação com *C. vulgaris* e as taxas de aplicação de melaço para peso final, comprimento, sobrevivência, fator de conversão alimentar, taxa de crescimento específico, consumo de água, taxa de eficiência proteica, tempo de sedimentação, comunidade planctônica e índices hematológicos. Os resultados indicaram que a alta taxa de aplicação de melaço (50%) no sistema de biofloco afeta o desempenho zootécnico, consumo de água, tempo de sedimentação e os índices hematológicos dos alevinos de tilápia do Nilo, prejudicando seu desenvolvimento. É recomendada a taxa de aplicação de melaço de 30% da alimentação diária para o cultivo de alevinos de tilápias em sistema de biofloco com baixa salinidade.

Palavras-chave: microalga; carboidrato; composição centesimal; desenvolvimento zootécnico; índices hematológicos.

INTRODUCTION

In 2016, world aquaculture production reached 80 million tons excluding aquatic plants, with inland fish aquaculture production representing 59.3% (FAO, 2018), among which the main species of fresh water aquaculture were the Nile tilapia *Oreochromis niloticus*. This fish is one of the most important species (4.2 million tons, representing 8% fish aquaculture) (FAO, 2018) due to their environmental resistance, rapid growth and salinity tolerance between 0 and 25 g L^{-1} (El-Sayed, 2006; Pereira et al., 2016).

Tilapia culture in brackish water is an alternative to Brazilian semi-arid zones, where water resources are limited and saline groundwater is the unique source of water available, with salinities between 1.1 and 10.0 g L^{-1} (Andrade Júnior et al., 2006; Brasil, 2012). Moreover, the desalination technology used for brackish groundwater results in highly saline residual water, which has a potential use for aquaculture (Soares et al., 2006).

Although aquaculture is considered a suitable alternative system for the increasing demand for animal-based protein, this activity has limitations in relation to water and land use. Therefore, intensive culture systems, such as biofloc technology (BFT), is a promising alternative (Emerenciano et al., 2017). BFT is based on carbon:nitrogen ratio management and minimum water exchange for production of microorganism biomass (Azim and Little, 2008; Avnimelech, 2012; Emerenciano et al., 2017), which results in higher productivity compared to conventional systems due to the elevated stocking density used (Avnimelech, 2015).

The carbon:nitrogen ratio is maintained through the addition of a source of organic carbon, which is used by bacteria to convert TAN (Total Ammoniacal Nitrogen) to bacterial biomass (Ebeling et al., 2006). Among the various sources of carbon, molasses is widely used because it stains water, reducing light penetration and associated algal growth, however, may present high level of impurities and content variability (Samocha et al., 2017). In addition, molasses is an inexpensive source of carbon used for various industrial fermentations (Miranda et al., 1996; Najafpour and Shan, 2003).

Some authors reported successfully culturing Nile tilapia in BFT (Long et al., 2015; Miranda-Baeza et al., 2017; Zapata-Lovera et al., 2017), including a study in brackish water (8 g L^{-1}) (Brol et al., 2017; Lima et al., 2018). However, due to the high stocking densities and minimal water exchange, nutrients accumulate, mainly nitrogen and phosphorus, in the biofloc culture (Krummenauer et al., 2011). About 60% of the nitrogen and 65% of the phosphorus that enter in the system are not converted into shrimp biomass (Silva et al., 2013). An alternative used to overcome the disadvantages is the use of microalgae in this system, due to their highly efficient removal of nitrogen and phosphorus (80-100%) in aquaculture, livestock and industry as have been reported by Prajapati et al. (2014), Posadas et al. (2015), Kuo et al. (2016) and Singh et al. (2017).

In addition, although the use of bioflocs serves to feed aquatic animals, it has low lipid content and therefore requires supplementation with natural foods, such as microalgae (Jung et al., 2017; Marinho et al., 2017). Inoculation of *C. vulgaris* together with *Scenedesmus obliquus*, in the culture system without water exchange, may improve the survival and development of cultured organisms (Jung et al., 2017). When cultured under mixotrophic conditions, *Chlorella* sp. showed higher lipid productivity (67-144 mg L^{-1} day^{-1}) (Yeh and Chang, 2012).

In this context, this study evaluated the effects of supplementation with *C. vulgaris* and two molasses application rates on planktonic community, zootechnical performance, proximate composition and hematological indices in low-salinity biofloc systems.

MATERIAL AND METHODS

Experimental conditions

All procedures were previously approved by the Ethics Committee on Animal Use of UFRPE under license number 129/2016. A 70-day indoor trial was conducted at the Sustainable Mariculture Laboratory (LAMARSU) of the Department of Fisheries and Aquaculture (DEPAq) of the Federal Rural University at Pernambuco (UFRPE), Recife, Brazil. The experiment had a 2 × 2 factorial design (Supplemented with *C. vulgaris* and molasses application rates) with the following treatments: BFT-C30 (Biofloc supplemented with *C. vulgaris* and molasses application rates of 30% of total daily feed); BFT-30 (Biofloc with molasses application rates of 30% of total daily feed); BFT-C50 (Biofloc supplemented with *C. vulgaris* and molasses application rates of 50% of total daily feed) and BFT-50 (Biofloc with molasses application rates of 50% of total daily feed), all in triplicate.

Five days prior to fish stocking, water from an indoor biofloc matrix tank (TAN 0.19 mg L^{-1}, $N\text{-}NO_2$ 0.12 mg L^{-1}, $N\text{-}NO_3$ 0.89 mg L^{-1}, alkalinity 160 mg $CaCO_3$ L^{-1}, pH 7.93, orthophosphate 1.76 mg L^{-1}, TSS 366 mg L^{-1}, SS 25 mL L^{-1} and salinity 10 g L^{-1}) was mixed and equally distributed to fill twelve experimental black-plastic rectangular tanks (50 L) up to ~50% of the volume, with the remaining volume filled with clean water (10 g L^{-1} salinity). The experimental units were maintained under constant aeration by using three cylindrical air stones (diameter 2.4 cm and length 2.6 cm) per tank. No water exchange was carried out during the experimental period, except for the addition of dechlorinated freshwater to compensate for evaporation losses. Light intensity was at ~ 1000 lux with a 12h light/12h dark regime.

Sugarcane molasses (30% organic carbon) was applied daily (10:00 a.m.) to each tank as a source of carbohydrates to promote the growth of heterotrophic bacteria. Molasses inputs were based on a percentage of the daily feed allotments (by weight) with application rates of 30% (C:N - 12:1) and 50% (C:N - 20:1) of the total daily feed. Calcium hydroxide ($Ca(OH)_2$) was added to maintain the alkalinity (> 100 mg L^{-1}) and the pH (> 7.5) in all treatments.

Supplemented with *C. vulgaris*

The microalgae *C. vulgaris* were obtained from Live Food Production Laboratory of DEPAq-UFRPE and cultures in a Provasoli medium. The culture was maintained at 25 ± 1 °C, salinity of 10 g L^{-1}, pH 7.9 and light intensity of ~2000 lux using a fluorescent lamp with a 24-h light photoperiod. The microalgae were supplemented every five days in the BFT-C30 and BFT-C50

treatments at a concentration of $5x10^4$ cell mL^{-1}, corresponding to an addition of approximately 250 mL of microalgae to the tanks, regardless of the waste from unconsumed *C. vulgaris*.

Water quality

Dissolved oxygen, temperature, salinity and pH (YSI model 556, Yellow Springs, Ohio, USA) were monitored twice a day (at 08:00 a.m. and 04:00 p.m.). Total ammonia nitrogen (TAN) (Koroleff, 1976), nitrogen-nitrite ($N\text{-}NO_2$) (Golterman et al., 1978), nitrogen-nitrate ($N\text{-}NO_3$) (Mackereth et al., 1978), orthophosphate (PO_4^{-3}) (APHA, 2005), alkalinity (mg $CaCO_3$ L^{-1}) (Felföldy et al., 1987), total suspended solids (TSS) (APHA, 2005 - 2540D) and volatile suspended solids (VSS) (APHA, 2005 - 2540E) were monitored weekly in the Limnology Laboratory of DEPAq-UFRPE. Alkalinity and dissolved nutrients were analyzed from effluent samples filtered with HAWP Millipore membranes of 0.45 µm pore size. For suspended solids the samples were filtered, dried (at 105 °C) and incinerated (at 550 °C) for inference of volatile suspended solids (volatile suspended solids = total suspended solids – inorganic suspended solids). The settleable solids (SS) were monitored three times per week by an Imhoff Cone (Avnimelech, 2012) and when its volume in the experimental tanks reached 50 mL L^{-1}, a settler was used in order to maintain SS values under this limit. The total time of use of the settler (ST) was also evaluated, using the equation:

$$ST\left(h\ Kg^{-1}\right) = Total\ time\ use\ of\ settler\ chamber\ (h)/Final\ biomass\ (Kg) \quad (1)$$

Microbial activity

The methods used to estimate microbial activity were those described by Vinatea et al. (2010). Once weekly, water from the tanks was collected at a depth of 10 cm and placed in triplicate sets of 200 mL glass bottles (clear or black). Initial and final oxygen concentrations were measured with an YSI 556 digital oxygen meter (Yellow Springs, Ohio, USA). Once initial oxygen was recorded, the bottles were sealed with glass stoppers held in place by plastic lids of the same color as the bottle.

The bottles were attached to a rotating table (QUIMIS Q225M) and incubated for 2 h, 2000 lux and at 200 rpm, a rotation sufficient to keep the particles of flocs in the water column. Gross primary production (GPP), net ecosystem production (NEP) and water column respiration (R) rates were recorded by the classic dark and light bottle method using the following formulae: gross primary production (GPP) (mg O_2 L^{-1} h^{-1}) = final O_2 of light bottle – final O_2 of dark bottle/time (h); net ecosystem production (NEP) (mg O_2 L^{-1} h^{-1}) = final O_2 of light bottle – initial O_2 of light bottle/time (h): water column respiration rate (R) (mg O_2 L^{-1} h^{-1}) = initial O_2 of dark bottle – final O_2 of dark bottle/time (h) (Strickland, 1960).

Cyanobacteria and phytoplankton monitoring

Vertical water sampling was performed at the start and weekly using plastic bottles of 500 mL for collection. The water was filtered through a cylindrical-conical 70 and 50 µm net mesh with the purpose of reducing the amount of suspended solids in the sample) and then it was filtered with a 15 µm mesh, for phytoplankton and cyanobacteria retention. The phytoplankton and cyanobacteria were fixed with formalin (4%), buffered with borax (1%) and stored in 2.5-mL plastic recipients. A Sedgewick-Rafter chamber and binocular optical microscope (Olympus CH30) with magnification of 400x were used for identification at the genus level, with the aid of identification keys (Hoek et al., 1995; Bicudo and Menezes, 2006), and quantification of the phytoplankton and cyanobacteria samples (Pereira-Neto et al., 2008).

Proximate composition

Analysis of crude protein, crude lipids, moisture content and ash contents whole body fish, biofloc samples and commercial feed were performed at the beginning and at the end of the experiment, in triplicate using standard methods (AOAC, 2012) at the Laboratory of Physical-Chemical Analysis of Foods of the UFRPE. Ten fish, at the beginning, and three fish in each tank, at the end, were randomly selected and macerated for the analyses. The biofloc samples (30g) were collected with cylindrical mesh net of 50 µm for retention of solids. The commercial feed (30g) were randomly selected and macerated for the analyses. Crude protein was determined by measuring nitrogen (N·x 6.25) using the Kjeldahl method and crude lipids was by the ether extraction method with the Soxhlet apparatus. The moisture content was determined by drying it at 105 °C for 18 hours until a stable weight was attained and the ash content was determined by incineration in a muffle at 550 °C.

Fish stocking, feeding and monitoring

The sex reversed male fingerlings of Nile tilapia *O. niloticus* (with 1.1 ± 0.4 g mean weight) were from commercial hatchery (Piscicultura Vale da Mina, Paulista, Pernambuco, Brazil), and stored in plastic bags with water for transportation to the laboratory. The fish were acclimatized for 5 days before being placed into experimental units. The fish were stocked in a 350 L tank with clean freshwater, at density of 625 fish m^{-3} and fed at 10% of fish wet biomass with commercial feed (36% crude protein) adjusted daily according to the estimated fish consumption. Each day 2 g L^{-1} of salinity was increased with marine water for acclimatization from freshwater until 10 g L^{-1} salinity within 5 days. After this acclimatization all fish were maintained at desired salinity for ten days.

The experimental units were stocked with Nile tilapia (3.15 ± 0.5g initial weight) at a density of 680 fish m^{-3} (34 shrimp per experimental unit). The fish fingerlings were fed four times a day (at 08:00 a.m., 11:00 a.m., 2:00 p.m. and 4:00 p.m.), with a commercial fish feed (36% crude protein, 4% crude fat, 5% crude fiber, 12% moisture and 3.276,1 kcal kg^{-1} of digestible energy). The daily feeding rate was 8% of body weight at the start of the experiment, gradually reduced to 5% of body weight at the end of the 70-day experiment based on the weekly biometrics.

Fish weight (BEL Engineering M503 – 0.001g) and length (ichthyometer) were monitored weekly (30% of population) in each experimental unit to determine biomass and survival. All fish

were counted weekly in each experimental unit for available survival. At the end of the experiment, biomass gain, final mean weight (W), final length, survival, feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), nitrogen retaining, yield, water consumption (WC) and sedimentation time (ST) were calculated, based on the following equations:

$$Biomass\ gain\ (g) = (Final\ weight\ (g) * Final\ population) - (Initial\ weight\ (g) * Initial\ population) \quad (2)$$

$$SGR\ (\%\ day^{-1}) = 100\ x\ \left[\begin{pmatrix} Ln\ Final\ weight\ (g) - \\ Ln\ Initial\ weight\ (g) \end{pmatrix} / Time\ (days) \right] \quad (3)$$

$$FCR = Feed\ supplied\ (g) / Biomass\ gain\ (g) \quad (4)$$

$$Survival\ (\%) = (Final\ population / Initial\ population)\ x\ 100 \quad (5)$$

$$Yield\ (kg\ m^{-3}) = Final\ biomass\ (kg) / Volume\ (m^3) \quad (6)$$

$$PER = Biomass\ gain\ (g) / Total\ protein\ intake\ (g) \quad (7)$$

$$Nitrogen\ retaining\ (\%) = \left[\begin{matrix} (final\ body\ nitrogen\ (g) - initial\ body\ nitrogen\ (g)) / \\ total\ nitrogen\ consumed\ (g) \end{matrix} \right] x\ 100 \quad (8)$$

$$WC\ (L\ Kg^{-1}) = Total\ water\ consumed\ (L) / Final\ biomass\ (Kg) \quad (9)$$

Hematological assays

At the end of the experiment, ten animals from each experimental unit were collected, anesthetized with eugenol (1.0 mL L^{-1}) and blood samples were collected from the caudal vessels by EDTA treated syringes. The hematological assays measured: hematocrit level (Goldenfarb et al., 1971); red blood cell count and mean corpuscular volume (MCV) (Wintrobe, 1934); and blood glucose levels using ACCU-CHEK ACTIVE blood glucose test meter (Roche), performed in the Aquatic Animal Health Laboratory of DEPAq-UFRPE. Moreover, during the weekly biometrics, external symptoms such as injuries, infection and other abnormal condition of fish body (integument and gills) were evaluated.

Statistical analysis

A two-way analysis of variance (ANOVA) was used to determine the effect of supplementation with *C. vulgaris* and molasses application rates of 30% and 50% of total daily feed and their interaction, after confirming homoscedasticity (Cochran $p < 0.05$) and normality (Shapiro-Wilk $p < 0.05$). Water quality variables, microbial activity, cyanobacteria and planktonic community were analyzed by performing repeated ANOVA measures. Tukey's test was used when differences between factors and treatments were detected ($p < 0.05$). Data analyses were performed using Statistica 10 software.

RESULTS

Water quality

The water quality variables temperature (27.7-27.9 °C), dissolved oxygen (5.3-5.4 mg L^{-1}), pH (7.7-7.8), orthophosphate (1.16-1.36 mg L^{-1}), TAN (0.44-0.48 mg L^{-1}), N- NO_2 (0.12-0.13 mg L^{-1}) and N- NO_3 (0.19-0.21 mg L^{-1}), were not significantly affected ($p > 0.05$) by supplementation with microalgae and molasses application rates (Table 1). However alkalinity levels significantly affected (p=0.01) by molasses application rates and their interaction (supplemented with microalgae and molasses application rates). Among the treatments, BFT-50 (165.08 ± 12.47 mg L^{-1}) and BFT-C50 (154.75 ± 9.94 mg L^{-1}) were higher compared with BFT-30 (120.48 ± 8.89 mg L^{-1}) and BFT-C30 (125.31 ± 10.33 mg L^{-1}).

The TSS (453-473 mg L^{-1}) and VSS (352-365 mg L^{-1}) were not significantly affected ($p > 0.05$) by supplementation with microalgae and molasses application rates (Table 1). However, SS levels significantly affected (p=0.02) by molasses application rates and their interaction (supplemented with microalgae and molasses application rates). Among the treatments, BFT-50 (54.95 ± 2.57 mL L^{-1}) and BFT-C50 (51.76 ± 2.82 mL L^{-1}) were higher compared with BFT-30 (43.40 ± 2.83 mL L^{-1}) and BFT-C30 (45.20 ± 2.62 mL L^{-1}). The ST were significantly affected (p=0.002) by molasses application rates and their interaction (Table 2). The molasses application rates of 50% of total daily feed were higher compared with molasses application rates of 30%.

Microbial activity

The GPP (-0.002-0.076 mg O_2 L^{-1} h^{-1}), NEP (-0.264-0.334 mg O_2 L^{-1} h^{-1}) and R (0.274-0.350 mg O_2 L^{-1} h^{-1}) were not significantly affected ($p > 0.05$) by supplemented with microalgae and molasses application rates (Table 3). There were fluctuations for GPP, NEP and R, however, the NEP presented negative values in all the treatments, throughout the experimental period, indicating that the environment was dominated by bacteria, even in the treatments where microalgae were added.

The phytoplankton community consisted of four genera at the beginning of the experiment (*Aphanocapsa* sp., *Chlorococcum* sp., *Cyclotela* sp. and *Tetraedron* sp.) and nine genera at the end (*Aphanocapsa* sp., *Chlorella* sp., *Chlorococcum* sp., *Chroococcus* sp., *Cyclotela* sp., *Cryptomonas* sp., *Oscilatoria* sp., *Rhabdonema* sp. and *Tetraedron* sp.).

The *Chlorella* sp. was the most abundant genera in all treatments, but due to its addition in BFT-C30 and BFT-C50 treatments, they had higher average levels of relative abundance, 55.99% and 57.54%, respectively, followed by BFT-30 and BFT-50 treatments, with 41.17% and 44.89%, respectively (Table 4). Although there was no significant difference between treatments, the supplementations with *C. vulgaris* added influenced the abundance of Chlorophyta (*Chlorella* sp., *Chlorococcum* sp. and *Tetraedron* sp.) and Cyanobacteria (*Aphanocapsa* sp., *Chroococcus* sp. and *Oscilatoria* sp.). The relative abundance of Cyanobacteria was higher in the treatments without supplemented with *C. vulgaris*, while the relative abundance of Chlorophyta was higher in the treatment with supplemented with *C. vulgaris*.

Table 1. Water quality variables of Nile tilapia fingerling cultivated in low-salinity biofloc system supplemented with *Chlorella vulgaris* and molasses application rates.

Variables	Treatments[1]				Significance (p value)[¥]		
	BFT-30	BFT-50	BFT-C30	BFT-C50	C	M	CxM
Temperature (°C)	27.74 ± 0.12	27.83 ± 0.11	27.90 ± 0.12	27.75 ± 0.10	ns	ns	ns
DO (mg L^{-1})	5.39 ± 0.04	5.45 ± 0.11	5.39 ± 0.04	5.33 ± 0.05	ns	ns	ns
Salinity (g L^{-1})	10.36 ± 0.11	10.29 ± 0.09	10.39 ± 0.12	10.35 ± 0.09	ns	ns	ns
TAN (mg L^{-1})	0.45 ± 0.06	0.45 ± 0.07	0.44 ± 0.06	0.48 ± 0.08	ns	ns	ns
N-nitrite (mg L^{-1})	0.12 ± 0.01	0.12 ± 0.02	0.12 ± 0.01	0.13 ± 0.02	ns	ns	ns
N-nitrate (mg L^{-1})	0.21 ± 0.03	0.19 ± 0.04	0.19 ± 0.02	0.19 ± 0.04	ns	ns	ns
PO_4^3 (mg L^{-1})	1.21 ± 0.07	1.16 ± 0.06	1.36 ± 0.09	1.16 ± 0.08	ns	ns	ns
Alkalinity (mg L^{-1})	120.48 ± 8.89^{b}	165.08 ± 12.47^{a}	125.31 ± 10.33^{b}	154.75 ± 9.94^{ab}	ns	*	*
pH	7.74 ± 0.04	7.81 ± 0.04	7.72 ± 0.05	7.80 ± 0.04	ns	ns	ns
SS (mL L^{-1})	43.40 ± 2.83^{b}	54.95 ± 2.57^{a}	45.20 ± 2.62^{b}	51.76 ± 2.82^{a}	ns	*	*
TSS (mg L^{-1})	453.47 ± 30.98	463.71 ± 27.65	461.42 ± 31.77	473.46 ± 28.12	ns	ns	ns
VSS (mg L^{-1})	352.82 ± 25.87	359.73 ± 24.00	359.09 ± 27.21	365.81 ± 24.78	ns	ns	ns

[1]The data correspond to the mean of thirty replicates ± standard deviation by treatments. Mean values on the same row with different superscript letters differ significantly ($p < 0.05$); C = supplemented with *C. vulgaris*; M = molasses application rates; CxM = supplemented with *C. vulgaris* and molasses application rates; ns = not-significant ($p > 0.05$); *$p < 0.05$; [¥]Results from split-plot two-way ANOVA and Tukey's test; DO = Dissolved oxygen; TAN = Total ammonia nitrogen; PO_4^3 = orthophosphate; SS = Settleable solids; TSS = Total suspended solids; VSS = Volatile suspended solids; BFT-C30 = Biofloc supplemented with *C. vulgaris* and molasses application rates of 30% of total daily feed; BFT-30 = Biofloc with molasses application rates of 30% of total daily feed; BFT-C50 = Biofloc supplemented with *C. vulgaris* and molasses application rates of 50% of total daily feed; and BFT-50 = Biofloc with molasses application rates of 30% of total daily feed, all in triplicate.

Table 2. Zootechnical performance of Nile tilapia fingerling cultured in low-salinity biofloc system supplemented with *Chlorella vulgaris* and molasses application rates.

Variables	Treatments[1]				Significance (p value)[¥]		
	BFT-30	BFT-50	BFT-C30	BFT-C50	C	M	CxM
Final Weight (g)	17.93 ± 0.20^{a}	12.05 ± 1.12^{b}	17.98 ± 0.85^{a}	13.86 ± 2.10^{ab}	ns	*	*
Final Length (cm)	9.73 ± 0.41^{a}	8.95 ± 0.32^{b}	9.88 ± 0.48^{a}	8.95 ± 0.57^{ab}	ns	*	*
Survival (%)	97.77 ± 2.22^{a}	87.77 ± 4.00^{b}	95.55 ± 2.93^{ab}	92.22 ± 1.11^{b}	ns	*	*
FCR	2.08 ± 0.03^{b}	4.96 ± 0.88^{a}	2.06 ± 0.17^{b}	3.74 ± 0.65^{a}	ns	*	*
SGR (% day^{-1})	2.91 ± 0.02^{a}	2.19 ± 0.16^{b}	2.91 ± 0.08^{a}	2.41 ± 0.26^{ab}	ns	*	*
PER	1.07 ± 0.01^{a}	0.59 ± 0.10^{b}	1.01 ± 0.06^{a}	0.80 ± 0.16^{ab}	ns	*	*
NR (%)	20,81 ± 0,76^{a}	10,22 ± 0,68^{b}	19,43 ±1,33^{a}	12,89 ±1,47^{b}	ns	*	*
Yield (kg m^{-3})	11.95 ± 0.10^{a}	7.34 ± 0.85^{b}	11.39 ± 0.59^{a}	9.05 ± 1.40^{ab}	ns	*	*
WC (L kg^{-1})	55.22 ± 7.13^{b}	93.28 ± 12.89^{a}	47.79 ± 6.41^{b}	51.26 ± 11.85^{b}	*	*	*
ST (h Kg^{-1})	23.47 ± 0.43^{b}	38.20 ± 3.25^{a}	24.44 ± 1.04^{b}	31.23 ± 4.59^{ab}	ns	*	*

[1]The data correspond to the mean of three ± standard deviation by treatments. Mean values on the same row with different superscript letters differ significantly ($p < 0.05$); C = supplemented with *C. vulgaris*; M = molasses application rates; CxM = supplemented with *C. vulgaris* and molasses application rates; ns = not-significant ($p > 0.05$); *$p < 0.05$; [¥]Results from split-plot two-way ANOVA and Tukey's test; FCR = Feed conversion ratio; SGR = Specific growth rate; PER = Protein efficiency ratio; NR = Nitrogen retaining; WC = water consumption; ST = sedimentation time. Treatments' abbreviations as in Table 1.

Table 3. Gross and net primary production and respiratory activity of Nile tilapia fingerling cultured in low-salinity biofloc system supplemented with *Chlorella vulgaris* and molasses application rates.

Variables	Treatments[1]				Significance (p value)[¥]		
(mg O_2 L^{-1} h^{-1})	BFT-30	BFT-50	BFT-C30	BFT-C50	C	M	CxM
GPP	-0.075 ± 0.114	-0.002 ± 0.140	-0.076 ± 0.114	-0.027 ± 0.097	ns	ns	ns
NEP	-0.312 ± 0.196	-0.264 ± 0.253	-0.334 ± 0.231	-0.316 ± 0.319	ns	ns	ns
R	0.274 ± 0.180	0.298 ± 0.183	0.285 ± 0.115	0.350 ± 0.199	ns	ns	ns

[1]The data correspond to the mean of thirty replicates ± standard deviation by treatments. Mean values on the same row with different superscript letters differ significantly ($p < 0.05$); C = supplemented with *C. vulgaris*; M = molasses application rates; CxM = supplemented with *C. vulgaris* and molasses application rates; ns = not-significant ($p > 0.05$); [¥]Results from split-plot two-way ANOVA and Tukey's test. GPP = Gross primary production; NEP = Net ecosystem production; R = Respiratory activity. Treatments' abbreviations as in Table 1.

Table 4. Relative abundance of the planktonic community and cyanobacteria of Nile tilapia fingerling cultured in low-salinity biofloc system supplemented with *Chlorella vulgaris* and two molasses application rates.

Division /Genera (%)	Initial	Treatments[1]				Significance (p value)[¥]		
		BFT-30	BFT-50	BFT-C30	BFT-C50	C	M	CxM
Chlorophyta	16.70	**49.82**	**55.92**	**65.51**	**65.47**	*	ns	ns
Tetraedron	7.10	0.96	0.68	0.33	0.22	ns	ns	ns
Chlorococcun	9.60	7.69	10.35	9.19	7.71	ns	ns	ns
Chlorella	0	41.17	44.89	55.99	57.54	*	ns	ns
Bacillariophyta	54.37	**18.80**	**15.93**	**11.21**	**12.77**	ns	ns	ns
Cyclotela	54.37	18.74	15.87	11.17	12.7	ns	ns	ns
Rhabdonema	0	0.06	0.06	0.04	0.07	ns	ns	ns
Cyanobacteria	28.92	**21.14**	**20.45**	**16.50**	**15.05**	*	ns	ns
Aphanocapsa	28.93	4.72	6.68	3.78	4.26	ns	ns	ns
Oscillatoria	0	1.42	4.95	6.24	3.46	ns	ns	ns
Chroococcus	0	15.03	8.82	6.48	7.33	*	ns	ns
Cryptophyta	0	**10.07**	**7.49**	**6.63**	**6.6**	ns	ns	ns
Cryptomonas	0	10.07	7.49	6.63	6.6	ns	ns	ns
TOTAL (cell mL^{-1})	8.02	3535.1	1810.31	2389.614	2657.25			

[1]The data correspond to the mean of thirty replicates by treatments. Mean values on the same row with different superscript letters differ significantly ($p < 0.05$); C = supplemented with *C. vulgaris*; M = molasses application rates; CxM = supplemented with *C. vulgaris* and molasses application rates; ns = not-significant ($p > 0.05$); *$p < 0.05$; [¥]Results from split-plot two-way ANOVA and Tukey's test. Treatments' abbreviations as in Table 1.

Table 5. Proximate composition (% dry weight) of whole body fish and biofloc from of Nile tilapia fingerling cultured in low-salinity biofloc system supplemented with *Chlorella vulgaris* and molasses application rates.

Proximate composition	Treatments[1]				Significance (p value)[¥]		
	BFT-30	BFT-50	BFT-C30	BFT-C50	C	M	CxM
Fish							
Moisture	74.55 ± 0.07^{b}	75.05 ± 0.99^{ab}	74.81 ± 0.33^{b}	75.77 ± 0.07^{a}	ns	ns	*
Crude protein	71.24 ± 1.88^{a}	64.74 ± 2.63^{b}	61.62 ± 1.04^{b}	63.31 ± 0.74^{b}	ns	*	*
Lipids	20.67 ± 0.59^{a}	16.86 ± 0.23^{b}	20.70 ± 0.38^{a}	16.51 ± 0.38^{b}	ns	*	*
Ash	7.97 ± 0.57	8.32 ± 0.10	7.56 ± 0.23	8.02 ± 2.06	ns	ns	ns
Biofloc							
Moisture	84.96 ± 3.11^{b}	89.06 ± 0.13^{a}	86.91 ± 0.11^{b}	89.16 ± 0.19^{a}	ns	ns	*
Crude protein	37.60 ± 1.17	34.19 ± 11.62	38.13 ± 1.42	36.81 ± 8.98	ns	*	ns
Lipids	1.75 ± 0.52	1.83 ± 0.13	1.24 ± 0.61	1.89 ± 0.07	ns	ns	ns
Ash	17.96 ± 1.05	17.18 ± 0.08	16.42 ± 0.74	17.11 ± 0.28	ns	ns	ns

[1]The data correspond to the mean of three ± standard deviation by treatments. Mean values on the same row with different superscript letters differ significantly ($p < 0.05$); C = supplemented with *C. vulgaris*; M = molasses application rates; CxM = supplemented with *C. vulgaris* and molasses application rates; ns = not-significant ($p > 0.05$); *$p < 0.05$; [¥]Results from split-plot two-way ANOVA and Tukey's test. Treatments' abbreviations as in Table 1.

Proximate composition

The moisture, crude protein and lipids content in fish were significantly affected ($p < 0.05$) by molasses application rates and its interaction with microalgae addition (Table 5). While for the biofloc content the crude protein was significantly affected ($p < 0.05$) by molasses application rates. The proximal composition found for commercial feed (8.38 ± 1.25% moisture, 35.56 ± 1.31% protein, 2.61 ± 0.36% lipid and 6.31 ± 0.68% of ash) was different from the information described on the label, especially lipids.

Tilapia zootechnical variables

All the zootechnical performance variables were significantly affected ($p < 0.05$) by molasses application rates and their interaction (Table 2). In the final weight, final length, survival, SGR, PER, nitrogen retaining, yield in the molasses application rates of 30% of total daily feed were higher compared with molasses application rates of 50%. However, FCR was lower in molasses application rates of 30% compared with 50%.

Table 6. Hematological indices of Nile tilapia fingerling cultivated in low-salinity biofloc system supplemented with *Chlorella vulgaris* and molasses application rates.

Variables	Treatments[1]				Significance (p value)[¥]		
	BFT - 30	BFT – 50	BFT – C30	BFT – C50	C	M	CxM
Hematocrit (%)	30.69 ± 2.84[a]	19.20 ± 2.50[b]	28.08 ± 0.90[a]	18.46 ± 2.39[b]	ns	*	*
Erythrocyte (x10^6 µL^{-1})	1.69 ± 0.04[a]	0.76 ± 0.17[b]	1.52 ± 0.11[a]	0.78 ± 0.08[b]	ns	*	*
MCV (fL)	256.59 ± 26.14	233.61 ± 22.34	217.14 ± 27.09	233.85 ± 11.94	ns	ns	ns
Glucose (mg dL^{-1})	53.27 ± 8.30[b]	77.46 ± 5.03[a]	53.60 ± 4.47[b]	51.33 ± 8.3[b]	ns	ns	*

[1]The data correspond to the mean of three ± standard deviation by treatments. Mean values on the same row with different superscript letters differ significantly ($p < 0.05$); C = supplemented with *C. vulgaris*; M = molasses application rates; CxM = supplemented with *C. vulgaris* and molasses application rates; ns = not-significant ($p > 0.05$); *$p < 0.05$; [¥]Results from split-plot two-way ANOVA and Tukey's test. MCV- Mean corpuscle volume. Treatments' abbreviations as in Table 1.

The WC were significantly affected (p=0.04) by supplementation with *C. vulgaris* and molasses application rates and their interaction between the factors (Table 2). In the treatments BFT-50 (93 L kg^{-1}) was higher compared with BFT-C50 (51.26 L kg^{-1}), BFT-30 (55.22 L kg^{-1}) and BFT-C30 (47.79 L kg^{-1}).

Hematological assays

The hematocrit levels and erythrocyte counts were significantly affected ($p < 0.05$) by molasses application rates and their interaction, and glucose was significantly affected ($p < 0.05$) by interaction between the factors; however MCV was not significantly affected (Table 6). In the molasses application rates of 30% of total daily feed the hematocrit levels and erythrocyte counts were higher compared with molasses application rates of 50%. From the fourth week of experiment, signs of stress were observed, such excess of superficial mucus and hemorrhage at the bases of the pectoral and caudal fins and mouth of the animals in the BFT-50 and BFT-C50 groups.

DISCUSSION

During the experiment, the variables of temperature and dissolved oxygen were maintained in ideal values for the cultivated Nile tilapia (El-Sayed, 2006). In heterotrophic systems, such as in the biofloc system, it is expected that the levels of inorganic nitrogen compounds would be kept low due to direct conversion into microbial biomass as found in another study (Ebeling et al., 2006). The daily addition of a carbohydrate source to stimulate the growth of heterotrophic bacteria contributed to the reduction of the nitrogen compounds of water (Emerenciano et al., 2017), furthermore, fluctuations did not occur in nitrogen (TAN, N- NO_2, N- NO_3) concentrations and may be related to the fact that the experiment started with a previously matured biofloc.

The orthophosphate levels increased throughout the rearing time and ranged from 1.08 to 1.45 mg L^{-1}. The low levels of orthophosphate compared with those found by Luo et al. (2014) (~12.3 mg L^{-1}) using the biofloc system, may be related to the abundance of the genera *Chlorella* (41-57% phytoplankton community) in all treatments. According to Ruiz et al. (2011) and Singh et al. (2017), *C. vulgaris* has a high potential for reducing the phosphorus of effluents.

In relation to alkalinity, biofloc systems lose buffering capacity and require frequent corrections (Azim and Little, 2008). The alkalinity levels were affected (p=0.01) by molasses application rates and their interaction. The BFT-50 and BFT-C50 treatments had higher alkalinity levels compared with BFT-30 and BFT-C30, probably in higher molasses application rates there is more heterotrophic microbial biomass in relation to the nitrifying bacteria. According Ebeling et al. (2006) the nitrification process promotes a more intense alkalinity reduction compared with the conversion of ammonium nitrogen into heterotrophic microbial biomass. The pH and alkalinity remained within the range considered adequate for tilapia cultivated by Avnimelech (2015) due to the addition of calcium hydroxide.

The mean values of settleable solids varied between 43 and 55 mL L^{-1} and were affected (p=0.02) by molasses application rates and their interaction. The higher molasses application rates (50% of the total daily feed, C:N 20:1) presented settleable solids values higher than 50 mL L^{-1}, in addition to denser and larger diameter bioflocs. Due to the high levels of solids, the treatments with higher molasses addition required a longer sedimentation time. Due to the use of the settler chamber, the values of SS and TSS were maintained at ideal levels by Avnimelech (2012), between 5 and 50 mL L^{-1} for SS and 200 and 500 mg L^{-1} for TSS. However, an increase of these values was observed throughout the rearing time, similar to that found by Long et al. (2015). The high concentrations of SS and TSS can cause discomfort in fish such as the accumulation of organic matter in the gills, which affect oxygen diffusion and also growth (Hargreaves, 2006; Avnimelech, 2012; Arantes et al., 2017). In a study of *Litopenaeus vannamei* culture in biofloc, Arantes et al. (2017) found that the use of a settler chamber promoted improved the zootechnical performance of shrimp, resulting in higher productivity, survival and final weight.

Based on the results of the net productivity, even supplemented with *C. vulgaris* the system presented negative values from the beginning of the experimental period. According to Avnimelech (2012), when using a C:N ratio greater than 10:1, it is possible to promote the succession and dominance of bacteria on the microalgae, thus corroborating the findings in the present study using the C:N ratio of 12:1 and 20:1, in treatments with 30%

and 50%, respectively. This high abundance of *Chlorella sp*. (55 - 57%) may have reduced the density of cyanobacteria in BFT-C30 and BFT-C50. This result is similar as observed by Turker et al. (2003) who verified that *Chlorella sp*. reduces the abundance of phytoplankton, especially cyanobacteria in Partitioned Aquaculture System (PAS). On the other hand, Malbrouck and Kestemont (2006), mentioned that the excess of cyanobacteria in the aquaculture system can cause problems to species. Moreover, Miranda-Baeza et al. (2017) observed that the incorporation of the genus *Oscillatoria* had a significant negative effect on the survival and growth performance (<90% and around 19g) of tilapia in the biofloc system, especially in treatments with greater abundance this genus.

The fish crude protein and lipids were affected ($p < 0.05$) by molasses application rates and their interaction. Jung et al. (2017) identified an influence of supplementation with *C. vulgaris* and *Scenedesmus obliquus* on the nutritional values (protein and lipids) of the tilapia cultivated in a biofloc system. However, in our study, we did not find effects just by supplementing with microalgae. This result may be related to the concentration and or frequency of supplemented microalgae. The values for proximal composition (53-80% crude protein and 4-27% lipids of the dry weight) of tilapia were similar to those reported by Azim and Little (2008), Luo et al. (2014) and Jung et al. (2017). The biofloc can also be used as a supplementary food source contributing close to 50% of protein requirement for fish (Avnimelech, 2007). The values for proximal composition (34-38% crude protein of the dry weight), were close to the values of protein content suggested for the diet in rearing tilapia (25 and 40%) (Craig and Helfrich, 2002; El-Sayed, 2006). The lipid content of the biofloc 1.67 ± 0.33% of the dry matter was relatively low, as were the results found by Jung et al. (2017). Several studies have reported low lipid levels in biofloc from fish (1.27 to 3.16% of dry matter) (Azim and Little, 2008; Luo et al., 2014). Low lipids levels in bioflocs may be related to low lipid content of the commercial feed used in this experiment (2.61% crude lipids).

Regarding the effect of salinity, there is an increase in energy expenditure as a function of osmotic regulation, since in fish, osmoregulation requires a high demand of metabolic energy, ranging from 20 to 50% of total energy expenditure, reducing the energy available for growth (Boeuf and Payan, 2001). Moreover, the low nutritional content of the commercial feed used probably influenced the reduced growth and higher FCR (2.03 to 5.86), due to the low lipid content found in the commercial feed (2.61%), different from the information described in the label. Lima et al. (2018) found similar values to those of the present study at a lower time (42 days), from 12.40 to 18.99g, 96.82 to 100% of the survival, and lower values of FCR (1.24-1.40) when cultivating tilapia at different densities in biofloc with 36% protein and 4% lipid feed. However, the difference in the feed conversion factor between treatments with 30% and 50% addition of molasses is due to the fact that the animals of the treatments with the highest application of molasses showed signs of stress. Fish exposed to stress situations presents changes in homeostasis, inducing changes in their physiological responses (Furuya, 2010). Even so, the yield values in BFT-30 and BFT-C30 were within the range recommended by Avnimelech (2015), between 10 to 40 kg fish m^{-3}.

The Zootechnical performance of Nile tilapia fingerlings was affected ($p < 0.05$) by molasses application rates and their interaction. However, the addition of microalgae *C. vulgaris* had no influence, as reported by Araújo et al. (2019), when evaluating different densities of *C. vulgaris* (2.5, 5 and $10x10^4$ cell mL^{-1}) in the culture of tilapia in biofloc with stocking density lower than the present study (250 fish m^{-3}), and found final weight of approximately 21 g and survival greater than 80%. The addition of *C. vulgaris* microalgae may not have influenced fish performance because it is already abundant in the culture system, as we found when evaluating the phytoplankton community, where *C. vulgaris* represented 55.99% (BFT-C30) and 57.54% (BFT-C50) in addition treatment, and 41.17% (BFT-30) and 44.89% (BFT-50) in treatments without addition.

The final weight and length, FCR, SGR, PER and yield were higher in BFT-30 and BFT-C30 as compared than BFT-50 and BFT-C50. Pérez-Fuentes et al. (2016), Zapata-Lovera et al. (2017) and Liu et al. (2018) observed a higher zootechnical performance of Nile tilapia fingerlings in C/N (10:1) ratios compared with higher C/N (20:1) ratios, as in the present study, where treatments with 50% addition of molasses presented lower final weight at the end of the cultivation. It is probable that the lower levels of solids (Pérez-Fuentes et al., 2016) and the dominance of the mix of microalgae, heterotrophic bacteria and autotrophic bacteria is more beneficial for fish and shrimp growth (Xu et al., 2016). Moreover, in high C:N ratios there may be consumption of large amounts of oxygen (Liu et al., 2018).

Due to the stress and reduction of feed intake observed in fish from treatments with 50% addition of molasses presented lower values of nitrogen retention, and consequently a lower rate of protein efficiency when compared to the other treatments (BFT-30 and BFT -C30). However, as nitrogen retention depends on the digestible energy content of feeds (Kaushik and Oliva Teles, 1985; Einen and Roem, 1997), the values found in the present study were lower than those reported by other studies with tilapia (>30%) (Furuya et al., 2005; Righetti et al., 2011).

The highest WC was from the BFT-50 treatment (93.28 ± 12.89 L kg^{-1}), while in the other treatments the consumption varied between 47.19-55.22 L kg^{-1}, showing a significant effect ($p < 0.05$) by supplemented with *C. vulgaris* and molasses application rates and their interaction. A similar result was found by Jung et al. (2017) where WC decreased about 82% when supplementation with two microalgae species were used. The WC was similar (52.48 L kg^{-1} with 500 fish m^{-3}) that reported by Lima et al. (2018) and lower compared with 750 to 1250 fish m^{-3} (76.32 to 101.54 L kg^{-1}).

A good indicator of fish health may be hematological indices (Martins et al., 2017). In the present study, stress caused by molasses application rates of 50% (C/N 20:1) of total daily feed (BFT-50 and BFT-C50) caused significant changes in the hematocrit level and the erythrocyte counts of the fish, resulting in macrocytic anemia. The values found in the treatments with molasses application rates of 30% of total daily feed (C:N 12:1) (BFT-30 and BFT-C30) remained in the ideal range described by several authors (20 to 32% for hematocrit and 1.9 to 5.0 x 10^6 μL^{-1} for erythrocyte counts) (Ueda et al., 1997; Signor et al., 2010; Costa et al., 2014; Martins et al., 2017). However, the values for treatments molasses application rates of 50% of total daily

feed (BFT-50 and BFT-C50) were below the recommended level (21-44% hematocrit level and 1.50 to 3.76 x 10^6 μL^{-1} erythrocyte count) (Tavares-Dias, 2015).

In addition, the values found for mean corpuscular volume (MCV) were higher than those described by Signor et al. (2010), Salvador et al. (2013) and Tavares-Dias (2015) for Nile tilapia, between 113.4 and 170 fL. This increase in MCV is the first response of fish in order to compensate for the decrease in the number of erythrocytes, by carrying larger amounts of hemoglobin (Tavares-Dias et al., 2002). Higher glycemia values were obtained in the treatments with molasses application rates of 50% of total daily feed and without supplemented microalgae. Although the values found were within the range described by Tavares-Dias (2015), between 14.1 and 92.1 mg dL^{-1}, the increase in glucose levels was reported in fish when associated with stressful conditions, with elevated levels of cortisol and high blood glucose due to the hyperglycemic characteristic of cortisol (Iwama et al., 1999).

The low quality of the feed used in the experiment influenced the performance of the fish, along with the high rate of addition of molasses. A high amount of molasses can cause serious physiological problems in fish (Azim and Little, 2008; De-Schryver et al., 2008), for these reasons, attention is needed when adding this carbon source to the system and using high C:N ratios and in the quality of the feed.

CONCLUSION

In summary, the data of the present study show that the interactions between supplementation with *C. vulgaris* and molasses application rates, influenced zootechnical performance, ST, WC, cyanobacteria density, fish proximal composition, hematocrit and erythrocyte levels of Nile tilapia fingerlings cultured in low-salinity biofloc system. However, the main factor was molasses application rates of 30% (C:N 12:1) of total daily feed. Future research should also address the use of different densities and/or the frequency of supplementation with microalgae, the species used and the nutritional role of phytoplankton in the tilapia biofloc culture system.

ACKNOWLEDGEMENTS

The authors are grateful to Tereza Santos (Limnology Laboratory-UFRPE) for their contribution to this study. We are also grateful for the financial support provided by the Brazilian National Counsil for Scientific and Technological Development (CNPq) for the aid granted to the Professor Alfredo Olivera Gálvez (PQ 311058/2015-9), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE).

REFERENCES

Andrade Júnior, A.S.; Silva, E.F.F.; Bastos, E.A.; Melo, F.B.; Leal, C.M. 2006. Uso e qualidade da água subterrânea para irrigação no semi-árido piauiense. Revista Brasileira de Engenharia Agrícola e Ambiental, 10(4): 873-880. **http://dx.doi.org/10.1590/S1415-43662006000400014**.

AOAC – Association of Official Analytical Chemists. 2012. Official methods of analysis. 19th ed. Gaithersburg: AOAC. 3000p.

APHA – American Public Health Association. 2005. Standard methods for the examination of water and wastewater. Washington: APHA. 560p.

Arantes, R.; Schveitzer, R.; Magnotti, C.; Lapa, K.R.; Vinatea, L. 2017. A comparison between water exchange and settling tank as a method for suspended solids management in intensive biofloc technology systems: effects on shrimp (*Litopenaeus vannamei*) performance, water quality and water use. Aquaculture Research, 48(4): 1478-1490. **http://dx.doi.org/10.1111/are.12984**.

Araújo, M.T.; Braga, I.F.M.; Cisneros, S.V.; Silva, S.M.B.C.; Gálvez, A.O.; Correia, E.S. 2019. The intensive culture of nile tilapia supplemented with the microalgae *Chlorella vulgar*is in a biofloc system. Boletim do Instituto de Pesca, 45(2): e398. **http://dx.doi.org/10.20950/1678-2305.2019.45.2.398**.

Avnimelech, Y. 2007. Feeding with microbial flocs by tilápia in minimal discharge bioflocs technology ponds. Aquaculture, 264(1-4): 140-147. **http://dx.doi.org/10.1016/j.aquaculture.2006.11.025**.

Avnimelech, Y. 2012. Biofloc technology: a practical guide book. 2nd ed. Louisiana: The Word Aquaculture Society. 272p.

Avnimelech, Y. 2015. Biofloc technology: a practical guide book. 3rd ed. Louisiana: The Word Aquaculture Society. 258p.

Azim, M.; Little, D. 2008. The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilápia (*Oreochromis niloticus*). Aquaculture, 283(1-4): 29-35. **http://dx.doi.org/10.1016/j.aquaculture.2008.06.036**.

Bicudo, C.E.; Menezes, M.A. 2006. Gêneros de algas continentais do Brasil: chave para identificação e descrições. 2ª ed. São Carlos: Rima. 502p.

Boeuf, G.; Payan, P. 2001. Review how should salinity influence fish growth? Comparative Biochemistry and Physiology Part C, 130(4): 411-423. http://dx.doi.org/**10.1016/S1532-0456(01)00268-X**. PMid:11738629.

Brasil, 2012. Programa Água Doce: documento base. Brasília: Ministério do Meio Ambiente (MMA). 324p.

Brol, J.; Pinho, S.M.; Sgnaulin, T.; Pereira, K.R.; Thomas, M.C.; Mello, G.L.; Miranda-Baeza, A.; Emerenciano, M.G.C. 2017. Tecnologia de bioflocos (BFT) no desempenho zootécnico de tilápias: efeito da linhagem e densidades de estocagem. Archivos de Zootecnia, 66(254): 229-235. http://dx.doi.org/**10.21071/az.v66i254.2326**.

Costa, D.V.; Ferreira, M.W.; Navarro, R.D.; Rosa, P.V.; Murgas, L.D.S. 2014. Parâmetros hematológicos de tilápias-do-Nilo (*Oreochromis niloticus*) alimentadas com diferentes fontes de óleo. Revista Brasileira de Saúde e Produção Animal, 15(3): 754-764. **http://dx.doi.org/10.1590/S1519-99402014000300023**.

Craig, S.; Helfrich, L.A. 2002. Understanding fish nutrition, feeds, and feeding. Virginia: Virginia Cooperative Extension. 4p. Publication 420-256.

De-Schryver, P.; Crab, R.; Defoirdt, T.; Boon, N.; Verstraete, W. 2008. The basics of bio-flocs technology: the added value for Aquaculture. Aquaculture, 277(3): 125-137. **http://dx.doi.org/10.1016/j.aquaculture.2008.02.019**.

Ebeling, J.M.; Timmons, M.B.; Bisogni, J.J. 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture, 257(1-4): 346-358. **http://dx.doi.org/10.1016/j.aquaculture.2006.03.019**.

Einen, O.; Roem, A.J. 1997. Dietary protein/energy ratios for Atlantic salmon in relation to fish size: growth, feed utilization and slaughter quality. Aquaculture Nutrition, 3(2): 115-126. **http://dx.doi.org/10.1046/j.1365-2095.1997.00084.x**.

El-Sayed, E.M. 2006. Tilapia culture. Wallingford: CABI Publishing. 277p. **http://dx.doi.org/10.1079/9780851990149.0000**.

Emerenciano, M.; Martínez-Córdova, L.R.; Martínez-Porchas, M.; Miranda-Baeza, A. 2017. Biofloc Technology (BFT): a tool for water quality management in aquaculture. In: Tutu, H. (Ed.). Water quality. New York: InTech. p. 91-109.

Felföldy, L.; Szabo, E.; Tothl, L. 1987. A biológiai vizminösités. Hungary: Vizügyi Hodrobiológia. 258p.

Food and Agriculture Organization of the United Nations – FAO. 2018. The state of world fisheries and aquaculture 2018: meeting the sustainable development goals. Rome: FAO. 227p.

Furuya, W.M. 2010. Tabelas brasileiras para a nutrição de tilapias. Toledo: GFM. 100p.

Furuya, W.M.; Botaro, D.; Macedo, R.M.G.; Santos, V.G.; Silva, L.C.R.; Silva, T.C.; Furuya, V.R.B.; Sales, P.J.P. 2005. Aplicação do conceito de proteína ideal para redução dos níveis de proteína em dietas para Tilápia-do-Nilo (*Oreochromis niloticus*). Revista Brasileira de Zootecnia, 34(5): 1433-1441. **http://dx.doi.org/10.1590/S1516-35982005000500002**.

Goldenfarb, P.B.; Bowyer, F.P.; Hall, E.; Brosious, E. 1971. Reproductibility in the hematology laboratory: the microhematocrit determination. American Journal of Clinical Pathology, 56(1): 35-39. **http://dx.doi.org/10.1093/ajcp/56.1.35**. PMid:5556212.

Golterman, H.J.; Clyno, R.S.; Ohnstad, M.A. 1978. Methods for physical and chemical analysis of freshwaters. London: Blackwell Scientific Publications. 213p.

Hargreaves, J.A. 2006. Photosynthetic suspended-growth systems in aquaculture. Aquacultural Engineering, 34(3): 344-363. **http://dx.doi.org/10.1016/j.aquaeng.2005.08.009**.

Hoek, C.; Mann, D.; Jahns, H.M. 1995. Algae: an introduction to Phycology. Cambridge: Cambridge University Press. 623p.

Iwama, G.K.; Vijayan, M.M.; Forsyth, R.B.; Ackerman, P.A. 1999. Heat shock proteins and physiological stress in fish. Integrative and Comparative Biology, 39(6): 901-909. http://dx.doi.org/**10.1093/icb/39.6.901**.

Jung, J.Y.; Damusaru, J.H.; Park, Y.; Kim, K.; Seong, M.; Je, H.W.; Kim, S.; Bai, S.C. 2017. Autotrophic biofloc technology system (ABFT) using *Chlorella vulgaris* and *Scenedesmus obliquus* positively affects performance of Nile tilapia (*Oreochromis niloticus*). Algal Research, 27(1): 259-264. **http://dx.doi.org/10.1016/j.algal.2017.09.021**.

Kaushik, S.J.; Oliva Teles, A. 1985. Effect of digestible energy on nitrogen and energy balance in rainbow trout. Aquaculture, 50(1-2): 89-101. **http://dx.doi.org/10.1016/0044-8486(85)90155-3**.

Koroleff, F. 1976. Determination of nutrients. In: Grasshoff, K. (Ed.). Methods of seawater analysis. New York: Verlag Chemie Weinhein. p. 117-187.

Krummenauer, D.; Peixoto, S.; Cavalli, R.O.; Poersch, L.H.; Wasielesky Junior, W. 2011. Superintensive cultue of white shrimp, *Litopenaeus vannamei*, in a biofloco technology system in shouter Brazil at different stocking densities. Journal of the World Aquaculture Society, 42(5): 726-733. **http://dx.doi.org/10.1111/j.1749-7345.2011.00507.x**.

Kuo, C.M.; Jian, J.F.; Lin, T.H.; Chang, Y.B.; Wan, X.H.; Lai, J.T.; Chang, J.S.; Lin, C.S. 2016. Simultaneous microalgal biomass production and CO2 fixation by cultivating *Chlorella* sp. GD with aquaculture wastewater and boiler flue gas. Bioresource Technology, 221(1): 241-250. **http://dx.doi.org/10.1016/j.biortech.2016.09.014**. PMid:27643732.

Lima, P.C.M.; Abreu, J.L.; Silva, A.E.M.; Severi, W.; Gálvez, A.O.; Brito, L.O. 2018. Nile tilapia fingerling cultivated in a low-salinity biofloc system at different stocking densities. Spanish Journal of Agricultural Research, 16(4): e0612. **http://dx.doi.org/10.5424/sjar/2018164-13222**.

Liu, G.; Zhu, S.; Liu, D.; Ye, Z. 2018. Effect of the C/N ratio on inorganic nitrogen control and the growth and physiological parameters of tilapias fingerlings, *Oreochromis niloticu* reared in biofloc systems. Aquaculture Research, 49(7): 2429-2439. **http://dx.doi.org/10.1111/are.13702**.

Long, L.; Yang, J.; Li, Y.; Guan, C.; Wu, F. 2015. Effect of biofloc technology on growth, digestive enzyme activity, hematology, and immune response of genetically improved farmed tilapia (*Oreochromis niloticus*). Aquaculture, 448(1): 135-141. **http://dx.doi.org/10.1016/j.aquaculture.2015.05.017**.

Luo, G.; Gao, Q.; Wang, C.; Liu, W.; Sun, D.; Li, L.; Tan, H. 2014. Growth, digestive activity, welfare, and partial cost-effectiveness of genetically improved farmed tilapia (*Oreochromis niloticus*) cultured in a recirculating aquaculture system and an indoor biofloc system. Aquaculture, 422-423(1): 1-7. **http://dx.doi.org/10.1016/j.aquaculture.2013.11.023**.

MacKereth, F.J.H.; Heron, J.; Talling, J.F. 1978. Water analysis: some revised methods for limnologists. London: Blackwell Scientific Publications. 120p.

Malbrouck, C.; Kestemont, P. 2006. Effects of microcystins on fish. Environmental Toxicology and Chemistry, 25(1): 72-86. **http://dx.doi.org/10.1897/05-029R.1**. PMid:16494227.

Marinho, Y.F.; Brito, L.O.; Silva Campos, C.V.F.; Severi, W.; Andrade, H.A.; Galvez, A.O. 2017. Effect of the addition of *Chaetoceros calcitrans, Navicula* sp. and *Phaeodactylum tricornutum* (diatoms) on phytoplankton composition and growth of *Litopenaeus vannamei* (Boone) postlarvae cultivated in a biofloc system. Aquaculture Research, 48(8): 4155-4416. **http://dx.doi.org/10.1111/are.13235**.

Martins, G.P.; Pezzato, L.E.; Guimarães, I.G.; Padovani, C.R.; Mazini, B.S.M.; Barros, M.M. 2017. Antinutritional factors of raw soybean on growth and haematological responses of Nile tilapia. Boletim do Instituto de Pesca, 43(2): 322-333. **http://dx.doi.org/10.20950/1678-2305.2017v43n3p322**.

Miranda, M.P.; Benito, G.G.; Cristobal, N.S.; Nieto, C.H. 1996. Color elimination from molasses wastewater by *Aspergillus niger.* Bioresource Technology, 57(3): 229-235. **http://dx.doi.org/10.1016/S0960-8524(96)00048-X**.

Miranda-Baeza, A.; Mariscal-López, M.A.; López-Elías, J.A.; Rivas-Vega, M.E.; Emerenciano, M.; Sánchez-Romero, A.; Esquer-Méndez, J.L. 2017. Effect of inoculation of the cyanobacteria *Oscillatoria* sp. on tilapia biofloc culture. Aquaculture Research, 48(9): 4725-4734. **http://dx.doi.org/10.1111/are.13294**.

Najafpour, G.D.; Shan, C.P. 2003. Enzymatic hydrolysis of molasses. Bioresource Technology, 86(1): 91-94. **http://dx.doi.org/10.1016/S0960-8524(02)00103-7**. PMid:12421015.

Pereira, D.S.P.; Guerra-Santos, B.; Moreira, E.L.T.; Albinati, R.C.B.; Ayres, M.C.C. 2016. Parâmetros hematológicos e histológicos de tilápia do nilo em resposta ao desafio de diferentes níveis de salinidade.

Boletim do Instituto de Pesca, 42(3): 635-647. **http://dx.doi.org/10.20950/1678-2305.2016v42n3p635**.

Pereira-Neto, J.B.; Dantas, D.M.M.; Gálvez, A.O.; Brito, L.O. 2008. Avaliação das comunidades planctônica e bentônica de microalgas em viveiros de camarão (*Litopenaeus vannamei*). Boletim do Instituto de Pesca, 34(4): 543-551.

Pérez-Fuentes, J.A.; Hernández-Vergara, M.P.; Pérez-Rostro, C.I.; Fogel, I. 2016. C:N ratios affect nitrogen removal and production of Nile tilapia *Oreochromis niloticus* raised in a biofloc system under high density cultivation. Aquaculture, 452(1): 247-251. **http://dx.doi.org/10.1016/j.aquaculture.2015.11.010**.

Posadas, E.; Morales, M.M.; Gomez, C.; Acién, F.G.; Muñoz, R. 2015. Influence of pH and CO2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways. Chemical Engineering Journal, 265(1): 239-248. **http://dx.doi.org/10.1016/j.cej.2014.12.059**.

Prajapati, S.K.; Choudhary, P.; Malik, A.; Vijay, V.K. 2014. Algae mediated treatment and bioenergy generation process for handling liquid and solid waste from dairy cattle farm. Bioresource Technology, 167(1): 260-268. **http://dx.doi.org/10.1016/j.biortech.2014.06.038**. PMid:24994683.

Righetti, J.S.; Furuya, W.M.; Conejero, C.I.; Graciano, T.S.; Vidal, L.V.O.; Michellato, M. 2011. Redução da proteína em dietas para tilápias-do-nilo por meio da suplementação de aminoácidos com base no conceito de proteína ideal. Revista Brasileira de Zootecnia, 40(3): 469-476. **http://dx.doi.org/10.1590/S1516-35982011000300002**.

Ruiz, J.; Álvarez, P.; Arbib, Z.; Garrido, C.; Barragán, J.; Perales, J.A. 2011. Effect of nitrogen and phosphorus concentration on their removal kinetic in treated urban wastewater by *Chlorella vulgaris*. International Journal of Phytoremediation, 13(9): 884-896. **http://dx.doi.org/10.1080/15226514.2011.573823**. PMid:21972511.

Salvador, R.; Claudiano, G.S.; Loureiro, B.A.; Marcusso, P.F.; Eto, S.F.; Pilarski, F.; Toazza, C.S.; Moraes, J.R.E.; Moraes, F.R. 2013. Desempenho e hematologia de tilápias-do-nilo alimentadas com dieta suplementada com *Saccharomyces cerevisiae*, vacinadas e desafiadas com *Streptococcus agalactiae*. Pesquisa Agropecuária Brasileira, 48(8): 892-898. **http://dx.doi.org/10.1590/S0100-204X2013000800012**.

Samocha, T.M.; Prangnell, D.I.; Hanson, T.R.; Treece, G.D.; Morris, T.C.; Castro, L.F.; Staresinic, N. 2017. Design and operation of super intensive, biofloc-dominated systems for indoor production of the Pacific White Shrimp, *Litopenaeus vannamei*: the Texas A&M AgriLife Research Experience. Louisiana: The World Aquaculture Society. 398p.

Signor, A.; Barros, M.M.; Pezzato, L.E.; Falcon, D.R.; Guimarães, I.G. 2010. Parâmetros hematológicos da tilápia-do-nilo: efeito da dieta suplementada com levedura e zinco e do estímulo pelo frio. Ciência Animal Brasileira, 11(3): 509-519. **http://dx.doi.org/10.5216/cab.v11i3.6016**.

Silva, K.R.; Wasielesky Junior, W.; Abreu. 2013. Nitrogen and Phosphorus Dynamics in the Biofloc Production of the Pacific White Shrimp, *Litopenaeus vannamei*. Journal of the World Aquaculture Society, 44(1): 30-41. **http://dx.doi.org/10.1111/jwas.12009**.

Singh, R.; Birru, R.; Sibi, G. 2017. Nutrient removal efficiencies of *Chlorella vulgaris* from urban wastewater for reduced eutrophication. Journal of Environmental Protection, 8(1): 1-11. **http://dx.doi.org/10.4236/jep.2017.81001**.

Soares, T.M.; Silva, I.J.O.; Duarte, S.N.; Silva, E.F.F. 2006. Destinação de águas residuárias provenientes do processo de dessalinização por osmose reversa. Revista Brasileira de Engenharia Agrícola e Ambiental, 10(3): 730-737. **http://dx.doi.org/10.1590/S1415-43662006000300028**.

Strickland, J. 1960. Measuring the production of marine phytoplankton. Ottawa: Fisheries Research Board of Canada. 172p. (Bulletin).

Tavares-Dias, M. 2015. Parâmetros sanguíneos de referência para espécies de peixes cultivados. In: Tavares-Dias, M.; Mariano, W.S. Aquicultura no Brasil: novas perspectivas. São Paulo: Pedro & João. p. 11-30.

Tavares-Dias, M.; Moraes, F.R.; Martins, M.L.; Santana, A.E. 2002. Haematological changes in *Oreochromis niloticus* Linnaeus, 1758 (Osteichthyes: Cichlidae) with Gill Ichthyophthiriasis and Saprolegniosis. Boletim do Instituto de Pesca, 28(1): 1-9.

Turker, H.; Eversole, A.G.; Brune, D.E. 2003. Filtration of green algae and cyanobacteria by Nile tilapia, *Oreochromis niloticus*, in the Partitioned Aquaculture System. Aquaculture, 215(1): 93-101. **http://dx.doi.org/10.1016/S0044-8486(02)00133-3**.

Ueda, I.K.; Egami, M.I.; Sassp, W.S.; Matushima, E.R. 1997. Estudos hematológicos em *Oreochromis niloticus* (Linnaeus, 1758) (Cichlidae, Teleostei). Parte I. Brazilian Journal of Veterinary Research and Animal Science, 34(5): 270-275. **http://dx.doi.org/10.11606/issn.2318-3659.v34i5p270-275**.

Vinatea, L.; Galvez, A.O.; Browdy, C.L.; Stokes, A.; Venero, J.; Haveman, J.; Lewis, B.L.; Lawson, A.; Shuler, A.; Leffler, J.W. 2010. Photosynthesis, water respiration and growth performance of *Litopenaeus vannamei* in a super-intensive raceway culture with zero water exchange: interaction of water quality variables. Aquacultural Engineering, 42(1): 17-24. **http://dx.doi.org/10.1016/j.aquaeng.2009.09.001**.

Wintrobe, M.M. 1934. Variations on the size and haemoglobin content of erythrocytes in the blood of various vertebrates. Folia Haematologica, 51(32): 32-49.

Xu, W.J.; Morris, T.C.; Samocha, T.M. 2016. Effects of C/N ratio on biofloc development, water quality, and performance of *Litopenaeus vannamei* juveniles in a biofloc-based, high-density, zero-exchange, outdoor tank system. Aquaculture, 453(1): 169-175. **http://dx.doi.org/10.1016/j.aquaculture.2015.11.021**.

Yeh, K.; Chang, J. 2012. Effects of cultivation conditions and media composition on cell growth and lipid productivity of indigenous microalga *Chlorella vulgaris* ESP-31. Bioresource Technology, 105(1): 120-127. **http://dx.doi.org/10.1016/j.biortech.2011.11.103**. PMid:22189073.

Zapata-Lovera, K.P.; Brito, L.O.; Lima, P.C.M.; Vinatea-Arana, L.A.; Galvez, A.O.; Cárdenas, J.M.V. 2017. Cultivo de alevines de tilapia en sistema biofloc bajo diferentes relaciones carbono/nitrógeno. Boletim do Instituto de Pesca, 43(3): 399-407. **http://dx.doi.org/10.20950/1678-2305.2017v43n3p399**.