Ciência e Agrotecnologia
SCIENCE AND AGROTECHNOLOGY
Ciência e Agrotecnologia, 47:e012722, 2023
http://dx.doi.org/10.1590/1413-7054202347012722
Animal Science and Veterinary
eISSN 1981-1829

Stocking density of red tilapia (*Oreochromis* sp.) reared in a commercial biofloc system in Colombia

Densidade de estocagem de tilápia vermelha (*Oreochromis* sp.) criada em sistema comercial de bioflocos na Colômbia

Daniel Leonardo Cala Delgado[1*], Jesaías Ismael da Costa[2], Fabiana Garcia Scaloppi[3]

[1]Universidad Cooperativa de Colombia, Animal Science Research Group, sede Bucaramanga, Bucaramanga, Colombia
[2]Universidade Nilton Lins, Programa de Pós-graduação em Aquicultura, Manaus, Amazonas, Brasil
[3]Universidade Estadual Paulista/Unesp, Centro de Aquicultura, Jaboticabal, SP, Brasil
*Corresponding author: daniel.cala@campusucc.edu.co
Received in September 20, 2022 and approved in April 27, 2023

ABSTRACT

Biofloc technology (BFT) has several advantages, such as low water supply requirements and apparent feed conversion ratio (FCR), and high densities. However, there is no information on adequate densities and their effect on the large-scale production of different species. In this study, we determined the effects of the stocking density of monosex red tilapia reared using BFT on the growth performance, water quality parameters, and chemical characteristics of fish on a large scale and under culture conditions in eastern Colombia. In total, six circular tanks (diameter: 14 m, height: 1 m, and capacity: 153 m³) were used in this investigation. Two stocking densities were considered: treatment 1 (T30: 30 fish/m³) and treatment 2 (T40: 40 fish/m³), in triplicate. The water quality parameters of the two treatment densities were within the normal ranges for the species, but dissolved oxygen decreased throughout the production process. The T30 and T40 treatments did not result in significant differences in growth performance. At the end of the trial, the FCRs were 1.6 and 1.7 from T30 and T40, respectively. Fishes raised at a lower density had a similar weight gain; however, their final biomass was not significantly different. BFT did not affect the nutritional characteristics of fish; the percentage of protein (%) in tilapia was 17.81% ±1% and 16.72% ±1% in the T30 and T40 groups, respectively. None of the fish were infected by *Salmonella* spp. or *Vibrio cholerae*.

Index terms: Aeration; flour; nutritional quality; innocuousness; aquaculture.

RESUMO

Tecnologia de bioflocos (BFT) tem vantagens em termos de redução do fornecimento de água, redução da taxa aparente de conversão alimenta FCR e aumento das densidades. Entretanto, não há informações sobre densidade adequadas e seu impacto na produção em largas escala das diferentes espécies. Este estudo teve como objetivo determinar os efeitos da densidade de povoamento da tilápia vermelha masculinizada no BFT sobre desempenho produtivo, parâmetros de qualidade da água e características químicas dos peixes em larga escala e sob condições de cultivo no leste da Colômbia. Foram usados seis tanques circulares de 14 m de diâmetro, com capacidade de 153-m³, as densidades estudadas foram tratamento 1 (T30—30 peixes/m³) e tratamento 2 (T40—40 peixes/m³), em triplicado. Os parâmetros de qualidade da água das duas densidades estavam dentro das faixas normais para a espécie, mas o oxigênio dissolvido tendia a diminuir durante todo o processo de produção. As densidades de estocagem de T30 e T40, respectivamente, não resultaram em diferenças significativas no desempenho de crescimento. No final da experiência, FCR eram 1,6 e 1,7 de T30 e T40, respectivamente. Os peixes produzidos com menor densidade tiveram um ganho de peso semelhante; entretanto, a biomassa final não apresentou diferenças significativas. O BFT não afetou as características nutricionais, a porcentagem (%) de proteína foi 17.81 ±1 e 16.72 ±1 para T30 e T40, respetivamente. Não há presença de bactérias *Salmonella* spp. e *Vibrio cholerae* na carne de tilápia.

Termos para indexação: Aeração; farinha; qualidade nutricional; inocuidade; aquicultura.

INTRODUCTION

In biofloc technology (BFT), bacteria convert fish waste into microbial biomass (Adineh et al., 2019), which serves as fish food and helps control nitrogen compounds and other nutrient levels, thus reducing the water use and feed conversion ratio (FCR) (Pérez-Fuentes et al., 2016). Such changes decrease water treatment costs compared to other production systems (De Schryver et al., 2008). An additional benefit of BFT is the increase in density by up to 20% than that in extensive systems (Khanjani; Sharifinia, 2020). However, to increase the density, the level of nitrogen compounds has to be controlled by inoculating microorganisms and supplying carbon sources (Crab et al., 2007).

High stocking density is often considered to be a stressor, which negatively affects the feeding (El-Hawarry et al., 2021), physiological response (Bañuelos-Vargas et al., 2021), growth, and survival (Manduca et al., 2020) of fish. Hence, studies on different production systems need to be conducted to determine the appropriate densities for improving BFT gains (Aliabad et al., 2022). Many studies have investigated the appropriate stocking density for tilapia cultured in BFT and found that the lower the density, the better the water quality parameters, growth, and survival (Ekasari; Maryam, 2012).

The BFT system has been extensively studied using the Nile tilapia (*Oreochromis niloticus*) under laboratory conditions and strictly controlled environments. Fingerlings of this species maintained at low (166 fish/m³) and medium (333 fish/m³) densities were heavier and survived better than fingerlings maintained at high densities of 600 fish/m³ (Liu et al., 2018b). A density of 33 fish/m³ is recommended to grow tilapia with an initial weight of 133 g, and they are expected to gain approximately 700 g (Manduca et al., 2021). For fishes weighing 50 g and grown for 84 days, a density of 40 fish/m³ provides better productive yields than densities of 20 fish/m³ and 60 fish/m³ (Zaki et al., 2020). A density of 50 fish/m³ showed the lowest feed conversion ratio for red tilapia (*Oreochromis* sp.), which did not increase even after 14 weeks in a small-scale experimental BFT system (Ekasari; Maryam, 2012; Ekasari et al., 2012).

Ekasari and Maryam (2012) reported a need for studies on monosex red tilapia (*Oreochromis* sp.) because uncontrolled reproduction interrupted the growth of the fish in their study. Thus, according to the suggestions of Ekasari and Maryam (2012) and Collazo-Lasso and Arias-Castellanos (2015), studies more specific to the conditions of the country and of each species need to be conducted. Therefore, in this study, we determined the effects of the stocking density of monosex red tilapia reared using BFT on the growth performance, water quality parameters, and chemical characteristics of large-scale fish culture in eastern Colombia.

MATERIAL AND METHODS

Study site

The study was conducted in the production facilities of Asociación Piscícola el Vergel (Asovergel) located in the municipality of Arauquita, Vereda la Arenosa (6°58' N, 71°07', and 143 m above the sea level).

Experimental design

In total, six circular tanks (diameter: 14 m, height: 1 m, and capacity: 153 m³) were used in this study. To avoid spillage, 5 cm of freeboard was left, as described by Deb, Noori and Rao (2020). Constant aeration was provided by 1.5-hp radial splash aerators.

This study was conducted using a randomized design and two stocking densities: treatment 1 (T30 with 30 fish/m³) and treatment 2 (T40 with 40 fish/m³), each with three replicates. The monosex tilapia used in the culture had an initial weight of 1 ±0.2 g. Feeding was conducted according to the feed manufacturer's recommendations. The study was performed under fish farm conditions during a 210-day production process.

The biofloc inoculum was formed in each tank following the methodology proposed by Shourbela et al. (2021). After the biofloc matured, the parameters were maintained as follows: cane molasses was dissolved in the water from the same tank, distributed homogeneously, and added periodically to avoid accumulation in a single area of the tank. Molasses containing 41.3% carbon was added as the carbon source at 15 times the total ammonia nitrogen concentration (TAN) to maintain a C:N ratio of 15:1 (Avnimelech, 1999). Calcium hydroxide (2–50 mg/L) was added daily to maintain an alkalinity of 120 ppm, following the recommendations of Martins et al. (2017). Settling solids (SSs) were monitored using 2,000-L plastic tanks (decanters) connected to the drainage of the production tanks through an airlift system. They were used once the SSs reached 50 mL/L, measured using the Imhoff cone.

Water consumption and quality

Water quality parameters were evaluated for each tank temperature (T). Dissolved oxygen concentration (DOC) and saturation (DOS) were measured using a YSI EcoSense® DO200A probe, and the pH was evaluated daily using a Hanna® Model 991300 device. Ammonium (NH_4-N), nitrite (NO_2-N), nitrate (NO_3-N), and alkalinity (A) were evaluated once a week using colorimetric methods established for each parameter (American Public Health Association - APHA, 2005). The Biofloc SSs were determined weekly using Imhoff cones according to the method described by Avnimelech (2009). Water consumption was determined following the methodology proposed by Das et al. (2022), where water was added only when the water level dropped by 5% due to evaporation.

Productive performance

Biometric samplings were conducted every 70 days, following the evaluations made during the production process, where the individual weight of 5% of the population was recorded. All fish were weighed together at the end of the production cycle and counted to calculate survival; then, the final biomass was calculated. The amount of food consumed per group was also recorded. The following equations were used for the zootechnical performance analysis, Survival (S, %) or the percentage of fish that survived until the end of each evaluation period was obtained using the following equation: S (%) = (Final number of fish)/(Initial number of fish) × 100; Average weight gain (AWG) was calculated as follows: AWG (g) = Final average weight − Initial average weight; Average feed intake (AFI) was calculated as follows: AFI (g/fish) = [Total food intake (g)]/(Number of fish); the FCR ratio between AFI and AWG was expressed as FCRR = [Amount of feed consumed (AFI)]/[weight gain (AWG)]; Final biomass (B) was calculated as follows: B (kg) = Final number of fish × Final average weight.

Proximal analysis of fish and biofloc

Five fish per tank were randomly collected for the proximal composition analysis, and they were analyzed according to AOAC procedures (Association of Official Analytical Chemists – AOAC, 2012). The samples were initially oven-dried at 110 °C to a constant weight and ground, and then, their composition was determined.

The SS samples were collected every three days during production by extracting them from the bottom of the settler of each tank by the drainage system. The settled and collected floccules were dried at room temperature and then stored. A sample of the mixture was used to measure moisture, ash, protein, fat, crude fiber, total carbohydrates, and caloric value, along with the proximal composition of micronutrients and macronutrients. The tests were conducted in triplicate. The macronutrients and micronutrients were evaluated via microwave digestion-assisted atomic absorption spectroscopy (NTC-EN 14084:2021).

Microbiological analysis of the fish

Five fish were collected per replicate for microbiological testing, which was conducted soon after the samples were obtained. The methodology proposed by Alsaggaf, Moussa and Tayel (2017) was used for determining bacterial load recovery. For this, 10 g of sample was collected from the back of each fish, deposited individually in 90 mL of sterilized saline solution (0.85% NaCl), and homogenized in a Stomacher® 400 Seward device. Then, serial dilutions were performed, and the diluted samples were plated on appropriate microbiological media. Microbiological counts were made in triplicate and recorded as CFU/g.

Statistical analysis

The data were analyzed using the R statistical program for Windows (version 3.6.3). All data initially expressed as a percentage were transformed into arcsine before conducting statistical analyses; non-transformed data are presented in tables and figures. The mean values between densities were compared by performing an independent sample t-test to evaluate the differences between T30 and T40 densities. The least significant difference test was conducted to compare the mean values for determining significant differences between factors. The proximal analysis of the biofloc was descriptive and not comparative.

Ethical consideration

This study was endorsed by the Cooperative University of Colombia Bioethics Subcommittee, with the endorsement code 008–202.

RESULTS AND DISCUSSION

Biofloc technology (BFT) is a limited or zero water exchange productive system, which ensures intensification and sustainable feeding management (Crab et al., 2012). Bioflocs are heterogeneous aggregates of suspended organic particles and associated microorganisms, including bacteria, fungi, flagellates, protozoans, ciliates, and algae (Luo et al., 2020). They are a rich source of proteins (Xu; Pan, 2012) and might be used as alternative ingredients to replace traditional protein sources used in small aquaculture production facilities (Hargreaves, 2013).

This study was conducted under commercial conditions and lasted the entire 30 weeks (210 days) of the production cycle. Our findings contributed to the information on the breeding of red tilapia in Colombia. Our results differed from those of other studies in that our study conditions were not controlled as they were in most published studies on this subject.

The final values of the water quality parameters are presented in Table 1. The differences between densities among the parameters measured during production were not significant. Similarly, in studies where Nile tilapia were maintained at densities of 800 fingerlings/m³ (average weight: 18 g) and evaluated in nurseries for 44 days, the water quality parameters were maintained; however, the production of decanting solids and biomass was more efficient at densities of 406 fish/m³ (Silva et al., 2022).

Table 1: A list of the water quality parameters for the biofloc red tilapia production system at densities of 30 fish/m³ and 40 fish/m³.

Parameter	T30	T40
Temperature (°C)	30.0±1.2	29.9±1.4
Dissolved oxygen (mg/L)	4.9±1.1	4.8±1.2
pH	7.0±0.4	6.9±0.4
NH_4–N (mg/L)	0.6±0.3	0.7±0.2
NO_2–N (mg/L)	0.5±0.1	0.5±0.1
NO_3–N (mg/L)	0.5±0.5	0.6±0.5
Alkalinity	201±7.0	198±5.0
SS (mL/L)	35.9±11.5	36.2±11

*SS = Settling Solids.

Performing adequate calculations for the aeration systems is necessary to ensure that at the end of large-scale production, oxygen levels do not reach lower limits, which can risk fish survival. The DOC (Figure 1) decreased as the production process progressed. The levels of NH4–N fluctuated throughout the production process and reached a maximum concentration of 1 mg/L in the two treatment densities, whereas the levels of NO_2–N and NO_3–N gradually increased in the two treatment densities. Controlling the water temperature in open-air systems and under farm conditions is difficult because it depends on environmental conditions. We found fluctuations between 27 °C and 32.1 °C for the two densities; this temperature range is within the recommended values for the species (Chervinski, 1982). The pH and alkalinity were maintained at suitable levels for production using BFT. The quantity of settling solids (SSs) increased as the production time progressed (Figure 2), starting at 20 mL/L and ending at an average of 50.4 mL/L and 51.6 mL/L for T30 and T40, respectively.

The results of the productive performance are presented in Table 2. The differences between the values obtained for the three biometrics performed during the production process were not significant.

After 210 days of production, the biomass was 2.332 ±20.4 kg and 2.879 ±14.2 kg, and 15.2 kg/m³ and 18.8 kg/m³ for T30 and T40, respectively. This occurred because more fish occurred in the T40 group; therefore, although the weight of each fish was lower, the total biomass was greater in the T40 group than in the T30 group. At the end of production, the average weight of fish was 494.4 ±13 g and 476.7 ±17.2 g in the T30 and T40 groups, respectively; the difference in the weight between the groups was not significant.

The chemical composition of red tilapia is presented in Table 3. The humidity was 76.23% and 77.01% in the T30 and T40 treatments, respectively. The protein content decreased as humidity increased, and the fat content was lower at higher densities, with values of 4.15% and 3.8% in the T30 and T40 groups, respectively.

The use of carbon sources, such as molasses, can increase biofloc production within a shorter period and increase beneficial microorganisms and the probiotic activity of microbial communities (Addo et al., 2023). The increase in heterotrophic microorganisms stimulated by the carbon source can prevent the proliferation of bacteria in the genus *Aeromonas* and *Vibrio* (Monroy-Dosta et al., 2013). The use of BFT excludes certain pathogenic bacteria through competition (Wu et al., 2012). The dominance of bacteria such as *Nitrospira* sp., *Nitrobacter* sp., and *Bacillus* sp. helps in effectively controlling pathogenic microorganisms (Monroy-Dosta et al., 2013). The microbiological composition of Red tilapia reared in a biofloc system at the study densities remained unaltered (Table 4). The microbial quantification showed that they were within the ranges allowed for human consumption, and *Salmonella* spp. and *V. cholerae* were absent in the evaluated samples. Therefore, we inferred that it is safe to consume red tilapia produced in biofloc systems at densities of 30–40 fish/m³, as microorganisms that cause foodborne diseases were undetected. Similar results were reported in a study where shrimp was cultured in biofloc systems. In that study, no sample was positive for *Salmonella* spp. or *Staphylococcus* coagulase, and the concentrations of *V. cholerae* and coliforms were low. These findings indicated that although fresh water was not added to the biofloc system, the development of undesirable microorganisms was controlled, and no adverse effects occurred on the final product (Brito et al., 2016).

The biofloc composition (Table 5) showed a protein content of 29.3% ±0.5% and a total carbohydrate content of 25.58% ±0.27% under the T30 treatment. The largest concentration of nutrients under the T30 treatment were calcium and nitrogen (6.96% ±0.1% and 3.03% ±0.07%, respectively).

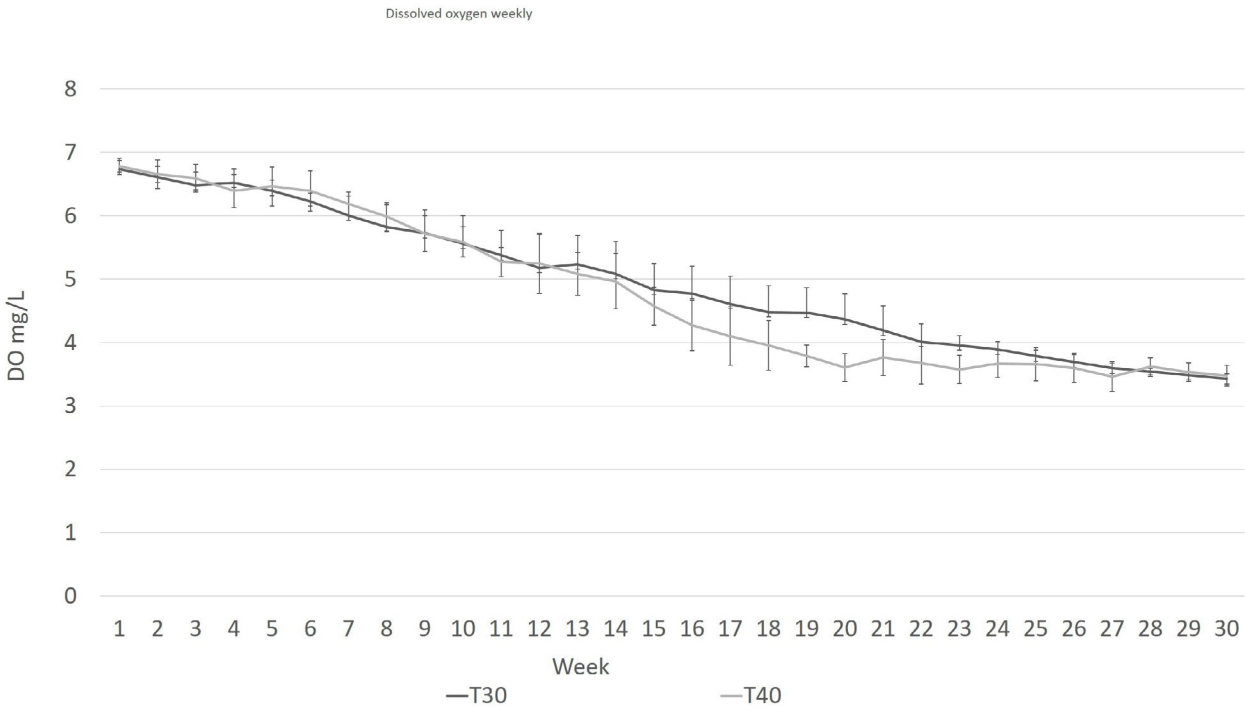

Figure 1: Variation in the dissolved oxygen concentrations with time in a red tilapia biofloc production system at different densities.

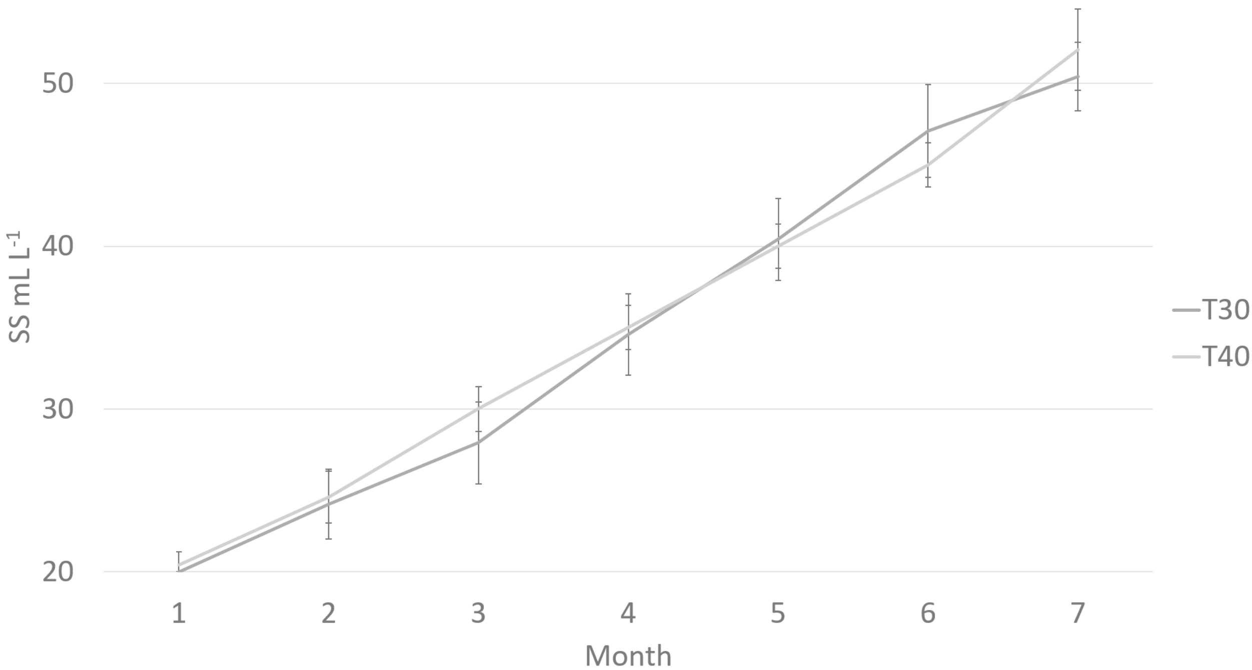

Figure 2: Settling solids in a red tilapia biofloc production system at different densities.

Table 2: Mean values (± standard deviation) of the zootechnical performance of tilapia after 30, 140, and 210 days of production in biofloc systems at different densities.

Days	Density	Zootechnical performance parameters				
		S (%)	AWG (g)	FC	FCR	B kg
70	T30	98.5±0.5	65.3±3.2	80±0.01	1.2±0.1	320
	T40	98.2±0.5	62.2±2.5	80±0.01	1.3±0.1	396
140	T30	98.1±0.4	259.4±7.2	344±0.01	1.3±0.1	1251
	T40	97.6±0.3	255.0±7.9	344±0.02	1.3±0.1	1589
210	T30	97.7±0.8	494.4±13	803±0.01	1.6±0.1	2332
	T40	97.1±1.3	476.7±17.2	799±0.02	1.7±0.1	2879

Note: The zootechnical performance parameters are: S (survival), AWG (average weight gain), FC (average feed consumption), and FCR (apparent feed conversion), B (biomass final period).

Table 3: Mean values (± standard deviation) of the centesimal composition (%) of red tilapia produced in a biofloc system at different densities.

Item (%)	T30	T40
Moisture	76.23±1	77.01±0.9
Ash	1.29±0.1	1.15±0.1
Fat	4.15±0.09	3.87±0.1
Protein	17.81±1	16.72±1
Total carbohydrates	0.49±0.02	0.38±0.03
Calories	136.34±3	130.18±3.5
Crude fiber	ND	ND

ND = Not detected.

Table 4: Microbiological analysis of the red tilapia grown in a biofloc system at different densities.

Analysis	Criterion or specifications	T30	T40
		Results	Results
E. coli	10–400 UFC/g	40	40
S. aureus coagulase-positive	100–1000 UFC/g	100	<100
Salmonella spp.	Absence/25 g	Absence	Absence
V. cholerae	Absence/25 g	Absence	Absence

Water exchange is minimized or avoided in biofloc systems, unlike traditional and cage systems (Avnimelech, 2015). In our study, water use was 89 L/kg and 72 L/kg of fish produced, under the T30 and T40 treatments, respectively, which showed that in BFT, increasing the density can enhance water use efficiency. Aquaculture technologies exhibit variation in water consumption. The use of water needs to be reduced to improve environmental control and reduce the release of nutrients into the environment. Among the methods available to fulfill this objective, the use of heterotrophic and nitrification bacteria, which process nitrogenous compounds and organic matter, is an efficient and promising strategy (Padeniya et al., 2022). For *Osteobrama belangeri* fingerlings, 70 and 80 fries/m^3 were better water use densities than 50 fries/m^3 (Das et al., 2022). This difference occurred probably because fish setups in the initial stages supported higher densities, but our study was conducted for a complete production cycle, and such initial effects soon disappeared.

In this study, only water lost by evaporation was replaced, as described in the study by Manduca et al. (2020) for Nile tilapia maintained under laboratory conditions. Here, the continuous supply of nutrients (provided by commercial feed, molasses, and calcium hydroxide) increased the accumulation of SS, an effect also described by Gaona et al. (2016) for the shrimp, *Litopenaeus vannamei*, cultured using a biofloc system.

Although settlers were used in our production system, SS increased during the production process, which was also observed by Deb, Noori and Rao (2020) in an empirical study on carp in a biofloc system. The flow of water can explain the increase in SS in the settlers (Gaona et al., 2016) and the low retention efficiency of the sedimentation tanks (Jung et al., 2020).

The gradual increase in SS in the two fish densities in this study could have increased the oxygen demand and the aerobic metabolism of organic matter. Additionally, the increase in biomass, along with the increase in SS, decreased DOC, which reached values of ~3 mg/L at the end of the production process. This was also found in a study by Ekasari and Maryam (2012), in which the DOC decreased as the biomass increased at different densities of red tilapia; however, they found no difference in the DOC between densities of 25, 50, and 100 fish/m³. Our results showed that red tilapia densities of 30 fish/m³ and 40 fish/m³ did not significantly affect DOC; however, they lowered DOC drastically. Therefore, the optimal aeration during the entire production process needs to be precisely calculated, depending on the densities, solids, and the aeration system used (Malpartida et al., 2018).

Table 5: Mean values (± standard deviation) of the proximate analysis and nutrients present in the biofloc meal in the red tilapia production system.

Bromatological proximate analysis		
Parameters	Value (%)	
	T30	T40
Moisture	12.1±0.1	11.3±0.3
Ash	28.8±0.5	27.5±0.2
Fat	0.38±0.03	0.37±0.01
Protein	29.3±0.5	30.7±0.4
Fiber	4.1±0.2	5.21±0.1
Total carbohydrates	25.3±0.27	25.58±0.3
Caloric value	236.51±23	233.14±11
Micro and macronutrient values (%)		
Nitrogen	3±0.02	3.02±0.01
Phosphorous	0.92±0.07	0.97±0.09
Potassium	0.29±0.05	0.31±0.03
Calcium	6.89±0.1	6.77±0.4
Magnesium	0.27±0.1	0.24±0.3
Manganese	0.01±0	0.01±0
Iron	0.63±0.01	0.64±0.02
Copper	0.01±0	0.01±0
Zinc	0.08±0	0.09±0
Boron	0.01±0	0.01±0
Sulfur	0.91±0	0.92±0

Regardless of the stocking density, water quality parameters usually remain unaffected and within the recommended ranges for tilapia production (Avnimelech, 2009; Monsees et al., 2017). Several studies have shown that an increase in density can lead to a decrease in oxygen levels and an increase or fluctuation in the levels of nitrogen compounds; the results of these studies matched those of our study (Ekasari; Maryam, 2012; Liu et al., 2018b; Wu et al., 2018; Deb; Noori; Rao, 2020; Manduca et al., 2021). Dissolved oxygen is the main limiting factor in fish production (Ngoepe et al., 2021), as well as, the production of NH_4–N and NO_2–N, which are highly toxic at an alkaline pH (Boyd; Tucker, 2012; Putra et al., 2020).

Nitrogen compounds accumulate as fish consume greater amounts of food (Ogbonna; Chinomso, 2010), as observed in our study; however, we found that densities of 30 fish/m³ and 40 fish/m³ did not affect the concentration of these compounds. Ekasari and Maryam (2012) reported that red tilapia cultivated in biofloc at densities of 100 fish/m³ did not have NH_4–N values that exceeded 1.1 mg L; the authors also found that NO_2–N levels remained stable during the 14 weeks of the study at the evaluated densities. Their findings differed from those obtained in this study, in which a gradual increase was observed.

In our study, NO_2–N was detected, although the recommended limit was not achieved. This occurred probably because molasses was used only when NH_4–N was ≥1 mg L. Similar results were obtained by Manduca et al. (2021), who reported a lower accumulation of SS and a reduction in costs by applying this strategy. Deb, Noori and Rao (2020) used the opposite strategy, replacing 50% of water when the concentration of NO_2–N exceeded 1 mg/L (Mahanand et al., 2013). This strategy is rarely recommended in biofloc systems because the microorganisms that participate in the transformation processes of nitrogen compounds are eliminated with each replacement (Liu et al., 2018a; Green et al., 2019).

The pH of the system remained stable throughout the red tilapia culture at the two evaluated densities; this was also reported in studies in which red tilapia were maintained at densities of 25, 50, and 100 fish/m³, where the pH ranged from 6.3 to 7.5 (Ekasari; Maryam, 2012). However, a study on the Nile tilapia reported a decrease in the pH at densities of 45 fish/m³ and 69 fish/m³ under laboratory conditions, where a biofloc system was used, but with partial replacement (Manduca et al., 2021).

Furtado et al. (2014) showed that alkalinity levels below 100 mg of $CaCO_3$ and pH values below 7 negatively affected the nitrification rates in biofloc systems, but pH values above 7.5 increased the toxicity due to the presence of nitrogen compounds in systems with zero water exchange. Therefore, in our study, calcium hydroxide ($Ca(OH)_2$) was supplied daily, as recommended for biofloc systems at 7.18% ±0.32% of feed consumption (Martins et al., 2017).

Density does not affect alkalinity when calculated based on the pH and when alkalinity is measured regularly to correct it and maintain its stability (Martins et al., 2017). This, in turn, helps maintain the stability of the pH and makes it immune to changes in density (Furtado; Poersch; Wasielesky, 2015).

An increase in stocking densities can decrease fish growth in different production systems, such as RAS (Ridha, 2006; Wu et al., 2018), cages (Garcia et al., 2013; Moniruzzaman et al., 2015), and biofloc systems (Haridas et al., 2017; Manduca et al., 2020; Zaki et al., 2020), regardless of the growth stage. Similar findings were also reported in a study where red tilapia was cultivated for 14 weeks in biofloc at a small scale.

In our study, the relationship between the weight of fish and the treatment densities was not significant. However, the fish in the T30 group were heavier throughout the production process, although we used to masculinize them, unlike the study conducted by Ekasari and Maryam (2012), which justified the interruption of growth in red tilapia. This occurred probably because of the stress generated by crowding, competition for food, and social dominance, which can modify the expenditure of metabolic energy reserved for growth and homeostasis (Martínez-Porchas et al., 2009; Manduca et al., 2021).

The increase in the density of tilapia can increase the time necessary for them to reach the harvest weights desired by producers (Garcia et al., 2013). In the biofloc systems, 180 fingerlings/m³ resulted in an FCR of 1.44, whereas 50 fingerlings/m³ resulted in an FCR of 1.21; these findings indicated that high densities could lead to an increase in this parameter (Liu et al., 2018a). We did not record such an increase, but feed conversion ratios (FCRs) of 1.6 and 1.7 were observed at T30 and T40, respectively.

Most studies on tilapia stocking densities that used BFT were generally conducted during a short growth period, mainly with the larval and juvenile stages of the Nile tilapia (Haridas et al., 2017; Lima et al., 2018; Liu et al., 2018b; Zaki et al., 2020; Manduca et al., 2020) or during the entire production phase, but on a small scale, in tanks with a capacity of less than 1,000 L (Manduca et al., 2021). A study was conducted with different densities of red tilapia in a small-scale biofloc system and with individuals of both sexes. The productive performance parameters were affected by uncontrolled fish reproduction in the system, but the densities used did not result in significant differences in the main parameters of productive performance (Ekasari; Maryam, 2012). These results of previous studies confirmed the results of our study, which was probably the first to evaluate densities of 30 and 40 fish/m³ of monosex red tilapia under culture conditions and at a large scale, using 153,000-L production tanks. The zootechnical performance parameters were not significantly different between treatment densities, but biomass was greater at the higher density, although each fish was lighter in that group. This occurred probably because of the presence of more fish; different densities might therefore be used depending on the demand for the weight of fish in the market.

The body composition of fresh red tilapia grown at two densities (30 and 40 individuals/m³) in a biofloc system was very similar to that reported by Silva et al. (2018) for the Nile tilapia at a density of 60 individuals/m³, where the average crude protein of fresh tilapia was found to be 14.3%, and the moisture content was 72.46%. *Piaractus brachypomus*, cultivated for 84 days in a biofloc system at a density of 42 fish/m³ and provided a commercial feed containing 25% crude protein, presented a body composition showing 74% moisture, 18% crude protein, and 1.2% ash (Díaz et al., 2020). The values obtained in that study matched the values obtained in the proximate analysis in this study.

In biofloc systems, microorganism concentrations increase throughout the production process (Monroy-Dosta et al., 2013). Microbiological communities in the digestive system and the culture water of shrimp (*Litopenaeus vannamei*) were found to increase with an increase in the stocking density in biofloc systems (Deng et al., 2019). However, we found that the concentrations of bacteria, such as *Staphylococcus aureus* and *E. coli*, were present in fresh tilapia but at safe concentrations, whereas, *Salmonella* spp. and *V. cholerae* were absent at the studied fish densities.

Biofloc flour has average crude protein levels ranging from 18.2% to 29.3% (Emerenciano et al., 2013; Minabi et al., 2020); we found similar protein levels in our study. These protein levels might be associated with heterotrophic bacteria that convert directly and rapidly accumulated ammonia into bacterial protein, which is reflected in the level of protein found in biofloc meal sediments (Ebeling et al., 2006).

This indicated that flour from biofloc SS is a protein alternative for fish feed, preferably in small-scale production systems, because the quantities of dry biofloc are not regular or sufficient for commercial-scale feed production (Delgado et al., 2020). Biofloc flour used at adequate levels in the diet of sea cucumbers (*Apostichopus japonicus*) improved growth by accelerating protein renewal (Chen et al., 2018).

Among all nutrients, calcium had the greatest concentration in the biofloc meal in our study, but its level was lower than that reported by Mabroke et al. (2019) for the biofloc meal used in the feed of Nile tilapia. Calcium levels might be linked to the continuous input of calcium hydroxide to maintain alkalinity, which is reflected in the sediment (Furtado et al., 2014). High calcium levels might also occur because of low quantities of available phosphorus (Pickens et al., 2020).

Nitrogen in biofloc flour is directly proportional to the floc size (Ekasari et al., 2014). About 88% of the accumulated nitrogen in a fish farming system is associated with food, and it accumulates in sediment at approximately 2.6% of nitrogen (Gross; Boyd; Wood, 2000). In our study, macronutrient concentrations (N, P, K, and Mg) were lower than that reported by Fimbres-Acedo et al. (2020), which often occurs with the micronutrient and macronutrient composition of effluents in different systems. Delaide et al. (2017) reported P, K, Fe, Mn, and S deficiency in sediments in a RAS system. The biofloc meal in this study was a pool collected throughout the production cycle; therefore, the results reflected the composition of the biofloc in different phases and under different management conditions of the production system, specifically the amounts of molasses and lime and the percentage of protein in the feed. Hence, compared to the findings of studies on fries where large amounts of carbon sources, carbonates, and high proteins were used, our results were less robust.

CONCLUSIONS

We did not find any effects on the physical and chemical parameters of red tilapia (*Oreochromis* spp.) reared in biofloc at densities of 30 and 40 fish/m³. The density of the fish did not affect productive performance indicators, but fish farmers should determine the weight of fish required by buyers to decide the stocking density to obtain fish that are ideal for selling. The biofloc system did not affect the nutritional characteristics or the microbiological concentrations of the fish.

ACKNOWLEDGMENTS

The source of funding for this review was the Universidad Cooperativa de Colombia and Dirección Nacional de Investigación DINAI – ID INV3183. The authors thank the Regional Head of Investigation Felipe Andres Gomez Velasquez for managing the project implementation processes.

AUTHOR CONTRIBUTION

Conceptual idea: Cala-Delgado, D. L.; Garcia Scaloppi, F.; Methodology design: Cala-Delgado, D. L.; Garcia Scaloppi, F.; Data collection: Cala-Delgado, D. L.; Costa, J.; Data analysis and interpretation: Cala-Delgado, D. L.; Garcia Scaloppi, F.; Costa, J.; and Writing and editing: Cala-Delgado, D. L.; Garcia Scaloppi, F.; Costa, J.

REFERENCES

ADDO, F. G. et al. Brown sugar addition enhanced nutrient removal rates, growth performance, and bacterial community in a rice straw-based biofloc shrimp culture system. Aquaculture, 567:739274, 2023.

ADINEH, H. et al. Biofloc technology improves growth, innate immune responses, oxidative status, and resistance to acute stress in common carp (*Cyprinus carpio*) under high stocking density. Fish & Shellfish Immunology, 95:440-448, 2019.

ALIABAD, H. S. et al. Effects of restricted feeding levels and stocking densities on water quality, growth performance, body composition and mucosal innate immunity of Nile tilapia (*Oreochromis niloticus*) fry in a biofloc system. Aquaculture, 546:737320, 2022.

ALSAGGAF, M. S.; MOUSSA, S. H.; TAYEL, A. A. Application of fungal chitosan incorporated with pomegranate peel extract as edible coating for microbiological, chemical and sensorial quality enhancement of Nile tilapia fillets. International Journal of Biological Macromolecules, 99:499-505, 2017.

AMERICAN PUBLIC HEALTH ASSOCIATION - APHA. Standard methods for the examination of water and wastewater. 21th ed. Washington, United States: American Public Health Association, American Water Works Association and Water Pollution Control Association - APHA, 2005. 8p.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. Official methods of analysis. 19th ed. Washington: AOAC. 22: 176-289, 2012.

AVNIMELECH, Y. Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture, 176(3-4):227-235,1999.

AVNIMELECH, Y. Biofloc technology: A practical guide book. World Aquaculture Society, Baton, Louisiana, United States. 175-200, 2009.

AVNIMELECH, Y. Biofloc technology: A practical guide book. 3rd Edition, The World Aquaculture Society, Baton, Louisiana, United States. 135: 230-255, 2015.

BAÑUELOS-VARGAS, I. et al. Antioxidant and immune response of juvenile red tilapia (*Oreochromis* sp) cultured at different densities in sea water with biofloc plus probiotics. Aquaculture, 544:737112, 2021.

BOYD, C. E.; TUCKER, C. S. Pond aquaculture water quality management. Springer Science & Business Media. 122: 56-68. 2012.

BRITO, C. et al. Microbiological quality of *Litopenaeus vannamei* culture using conventional and biofloc systems. Aquaculture Research, 47(10):3098-3108, 2016.

CHEN, J. et al. Effects of dietary biofloc on growth, digestibility, protein turnover and energy budget of sea cucumber *Apostichopus japonicus* (Selenka). Animal Feed Science and Technology, 241:151-162, 2018.

CHERVINSKI, J. Environmental physiology of tilapias. *In*: PULLIN, R. S. V.; LOWE-MCCONNELL, R. H. The biology and culture of tilapias. International Center for Living Aquatic Resources Management: Manila, Philippines. p.119-128, 1982.

COLLAZOS-LASSO, L. F.; ARIAS-CASTELLANOS, J. A. Fundamentals of bioflocs technology (BFT). An alternative for fish farming in Colombia. A review. Orinoquia, 19(1):77-86, 2015.

CRAB, R. et al. Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture, 270(1-4):1-14, 2007.

CRAB, R. et al. Biofloc technology in aquaculture: Beneficial effects and future challenges. Aquaculture, 356:351-356, 2012.

DAS, P. C. et al. Growth performance and immune responses of pengba (*Osteobrama belangeri*) during high-density fingerling rearing in biofloc system. Aquaculture Research, 53(17):6378-6388, 2022.

DE SCHRYVER, P. et al. The basics of bio-flocs technology: The added value for aquaculture. Aquaculture, 277(3-4):125-137, 2008.

DEB, S.; NOORI, M. T.; RAO, P. S. Application of biofloc technology for Indian major carp culture (polyculture) along with water quality management. Aquacultural Engineering, 91:102106, 2020.

DELAIDE, B. et al. Plant and fish production performance, nutrient mass balances, energy and water use of the PAFF Box, a small-scale aquaponic system. Aquacultural Engineering, 78:130-139, 2017.

DELGADO, D. L. C. et al. Proximal and sensory analysis of red tilapia (*Oreochromis* sp.) fed with fish tanks sediments from a Biofloc culture. Food Science and Technology, 41(4):870-876, 2020.

DENG, Y. et al. Effect of stock density on the microbial community in biofloc water and Pacific white shrimp (*Litopenaeus vannamei*) gut microbiota. Applied Microbiology and Biotechnology, 103:4241-4252, 2019.

DÍAZ, H. A. A. et al. Organoleptic and nutritional characteristics of fillets of pirapitinga fed different protein sources in a biofloc system. Pesquisa Agropecuária Brasileira, 55:e01795, 2020.

EBELING, J. M. et al. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. Aquaculture, 257(1-4):346-358, 2006.

EKASARI, J.; MARYAM, S. Evaluation of biofloc technology application on water quality and production performance of red tilapia *Oreochromis* sp. cultured at different stocking densities. Hayati Journal of Biosciences, 19(2):73-80, 2012.

EKASARI, J. et al. The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. Aquaculture, 426:105-111, 2014.

EL-HAWARRY, W. N. et al. The influence of carbon source on growth, feed efficiency, and growth-related genes in Nile tilapia (*Oreochromis niloticus*) reared under biofloc conditions and high stocking density. Aquaculture, 542:736919, 2021.

EMERENCIANO, M. et al. Evaluation of biofloc technology in pink shrimp *Farfantepenaeus duorarum* culture: Growth performance, water quality, microorganisms profile and proximate analysis of biofloc. Aquaculture International, 21(6):1381-1394, 2013.

FIMBRES-ACEDO, Y. E. et al. Hydroponic horticulture using residual waters from *Oreochromis niloticus* aquaculture with biofloc technology in photoautotrophic conditions with Chlorella microalgae. Aquaculture Research, 51(10):4340-4360, 2020.

FURTADO, P. S. et al. Application of different doses of calcium hydroxide in the farming shrimp *Litopenaeus vannamei* with the biofloc technology (BFT). Aquaculture International, 22(3):1009-1023, 2014.

FURTADO, P. S.; POERSCH, L. H.; WASIELESKY, W. The effect of different alkalinity levels on *Litopenaeus vannamei* reared with biofloc technology (BFT). Aquaculture International, 23:345-358, 2015.

GAONA, C. A. P. et al. Biofloc management with different flow rates for solids removal in the *Litopenaeus vannamei* BFT culture system. Aquaculture International, 24(5):1263-1275, 2016.

GARCIA, F. et al. Stocking density of Nile tilapia in cages placed in a hydroelectric reservoir. Aquaculture, 410-411:51-56, 2013.

GREEN, B. W. et al. E. Effects of dietary protein content on hybrid tilapia (*Oreochromis aureus*× *O. niloticus*) performance, common microbial off-flavor compounds, and water quality dynamics in an outdoor biofloc technology production system. Aquaculture, 503:571-582, 2019.

GROSS, A.; BOYD, C. E.; WOOD, C. W. Nitrogen transformations and balance in channel catfish ponds. Aquacultural Engineering, 24(1):1-14, 2000.

HARGREAVES, J. Á. Biofloc production systems for aquaculture (SRAC Publication, No 4503). Stoneville: Southern Regional Aquaculture Center Publication. 2013. 12p.

HARIDAS, H. et al. Enhanced growth and immuno-physiological response of genetically improved farmed tilapia in indoor biofloc units at different stocking densities. Aquaculture Research, 48(8):4346-4355, 2017.

JUNG, J. Y. et al. Evaluation of floc-harvesting technologies in biofloc technology (BFT) system for aquaculture. Bioresource Technology, 314:123719, 2020.

KHANJANI, M. H.; SHARIFINIA, M. Biofloc technology as a promising tool to improve aquaculture production. Reviews in Aquaculture, 12(3):1836-1850, 2020.

LIMA, E. C. R. D. et al. Culture of Nile tilapia in a biofloc system with different sources of carbon. Revista Ciência Agronômica, 49(3):458-466, 2018.

LIU, G. et al. Influence of stocking density on growth, digestive enzyme activities, immune responses, antioxidant of *Oreochromis niloticus* fingerlings in biofloc systems. Fish & Shellfish Immunology, 81:416-422, 2018a.

LIU, W. et al. Effect of no carbohydrate addition on water quality, growth performance and microbial community in water-reusing biofloc systems for tilapia production under high-density cultivation. Aquaculture Research, 49(7):2446-2454, 2018b.

LUO, G. et al. Effects of carbohydrate addition strategy and biofloc levels on the establishment of nitrification in biofloc technology aquaculture systems. Aquaculture, 514:734441, 2020.

MABROKE, R. S. et al. Floc meal as potential substitute for soybean meal in tilapia diets under biofloc system conditions. Journal of Oceanology and Limnology, 37:313-320, 2019.

MAHANAND, S. S.; MOULICK, S.; RAO, P. S. Water quality and growth of rohu, *Labeo rohita*, in a biofloc system. Journal of Applied Aquaculture, 25(2):121-131, 2013.

MALPARTIDA PASCO, J. J. et al. Production of Nile tilapia *Oreochromis niloticus* grown in BFT using two aeration systems. Aquaculture Research, 49(1):222-231, 2018.

MANDUCA, L. G. et al. Effects of a zero exchange biofloc system on the growth performance and health of Nile tilapia at different stocking densities. Aquaculture, 521: 735064, 2020.

MANDUCA, L. G. et al. Effects of different stocking densities on Nile tilapia performance and profitability of a biofloc system with a minimum water exchange. Aquaculture, 530:735814, 2021.

MARTÍNEZ-PORCHAS, M. et al. Cortisol and glucose: Reliable indicators of fish stress?. Pan-American Journal of Aquatic Sciences, 158-178, 2009.

MARTINS, G. B. et al. The utilization of sodium bicarbonate, calcium carbonate or hydroxide in biofloc system: Water quality, growth performance and oxidative stress of Nile tilapia (*Oreochromis niloticus*). Aquaculture, 468:10-17, 2017.

MINABI, K. et al. Effects of different carbon to nitrogen ratios in the biofloc system on water quality, growth, and body composition of common carp (*Cyprinus carpio* L.) fingerlings. Aquaculture International, 28:1883-1898, 2020.

MONIRUZZAMAN, M. et al. Effects of stocking density on growth, body composition, yield and economic returns of monosex tilapia (*Oreochromis niloticus* L.) under cage culture system in Kaptai Lake of Bangladesh. Journal of Aquaculture Research & Development, 6(8):1000357, 1, 2015.

MONROY-DOSTA, M. D. C. et al. Microbiology community composition and abundance associated to biofloc in tilapia aquaculture. Revista de Biología Marina y Oceanografía, 48(3):511-520, 2013.

MONSEES, H. et al. Chronic exposure to nitrate significantly reduces growth and affects the health status of juvenile Nile tilapia (*Oreochromis niloticus* L.) in recirculating aquaculture systems. Aquaculture Research, 48(7):3482-3492, 2017.

NGOEPE, T. K. et al. The effects of dissolved oxygen and dietary protein levels on growth performance, physiological parameters and the immune response of the genetically improved farmed tilapia juveniles (*Oreochromis niloticus*). Aquaculture Research, 52(2):547-558, 2021.

OGBONNA, J.; CHINOMSO, A. Determination of the concentration of ammonia that could have lethal effect on fish pond. Journal of Engineering and Applied Sciences 5:1-5, 2010.

PADENIYA, U. et al. Microbial interactions, growth, and health of aquatic species in biofloc systems. Water, 14(24):4019, 2022.

PÉREZ-FUENTES, J. A. et al. C: N ratios affect nitrogen removal and production of Nile tilapia *Oreochromis niloticus* raised in a biofloc system under high density cultivation. Aquaculture, 452:247-251, 2016.

PICKENS, J. M. et al. Integrating greenhouse cherry tomato production with biofloc tilapia production. Horticulturae, 6(3):44, 2020.

PUTRA, I. et al. Effect of different biofloc starters on ammonia, nitrate, and nitrite concentrations in the cultured tilapia *Oreochromis niloticus* system. F1000Research, 9:293, 2020.

RIDHA, M. T. Comparative study of growth performance of three strains of Nile tilapia, *Oreochromis niloticus*, L. at two stocking densities. Aquaculture Research, 37(2):172-179, 2006.

SHOURBELA, R. M. et al. The effect of stocking density and carbon sources on the oxidative status, and nonspecific immunity of Nile tilapia (*Oreochromis niloticus*) reared under biofloc conditions. Animals, 11(1):184, 2021.

SILVA, B. C. D. et al. Tilapia nursery stocking densities in a chemoautotrophic biofloc system. Ciência e Agrotecnologia, 46:e022321, 2022.

SILVA, M. A. et al. Crude protein levels in diets for two growth stages of Nile tilapia (*Oreochromis niloticus*) in a biofloc system. Aquaculture Research, 49(8):2693-2703, 2018.

WU, L. et al. Effect of wastewater COD/N ratio on aerobic nitrifying sludge granulation and microbial population shift. Journal of Environmental Sciences, 24(2):234-241, 2012.

WU, F. et al. Effect of stocking density on growth performance, serum biochemical parameters, and muscle texture properties of genetically improved farm tilapia, *Oreochromis niloticus*. Aquaculture International, 26(5):1247-1259, 2018.

XU, W. J.; PAN, L. Q. Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. Aquaculture, 356-357:147-152 2012.

ZAKI, M. A. et al. The impact of stocking density and dietary carbon sources on the growth, oxidative status and stress markers of Nile tilapia (*Oreochromis niloticus*) reared under biofloc conditions. Aquaculture Reports, 16:100282, 2020.