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Water salinity during masculinization of Nile tilapia in biofloc system

Abstract – The objective of this work was to evaluate the effect of different water salinity levels on the growth performance, survival, and masculinization rate of Nile tilapia (*Oreochromis niloticus*) larvae in a biofloc technology (BFT) system. Seven salinity levels (0, 2, 4, 6, 8, 10, and 12 g L^{-1}) were tested during four weeks in the masculinization period after the absorption of the yolk sac in a matured biofloc system. The water quality variables were within the recommended range for Nile tilapia farming. However, the nitrite peaks were higher at higher salinity levels and were associated with the lower survival of fish at salinity levels equal to or higher than 6 g L^{-1}. There was no difference between treatments for average final body weight and masculinization rate. Final biomass and survival decreased, and the feed offered as a proportion of final biomass showed the worst results because of the increase in salinity. Therefore, since higher masculinization rates are not obtained at slight and moderate levels of saline water, salinity should be kept close to 0 g L^{-1} for the masculinization protocol of Nile tilapia in a BFT, for a better survival and higher biomass of the fish, as well as a lower waste of the offered feed.

Index terms: *Oreochromis niloticus*, BFT, sexual inversion, sodium chloride.

Salinidade da água durante a masculinização de tilápia-do-nilo em sistema de bioflocos

Resumo − O objetivo deste trabalho foi avaliar o efeito de diferentes níveis de salinidade da água sobre o desempenho de crescimento, a sobrevivência e a taxa de masculinização de larvas de tilápia-do-nilo (*Oreochromis niloticus*) em sistema de tecnologia de bioflocos (BFT). Sete níveis de salinidade (0, 2, 4, 6, 8, 10 e 12 g L^{-1}) foram testados durante quatro semanas no período de masculinização após a absorção do saco vitelino, em sistema de bioflocos maturados. As variáveis de qualidade da água estiveram dentro dos intervalos recomendados para a produção de tilápia-do-nilo. Entretanto, os picos de nitrito foram mais altos nos tratamentos com maiores níveis de salinidade e foram associados à menor sobrevivência dos peixes em salinidade igual ou superior a 6 g L^{-1}. Não houve diferença entre os tratamentos quanto ao peso médio final e à taxa de masculinização. A biomassa final e a sobrevivência diminuíram, e o alimento oferecido como proporção da biomassa final apresentou os piores resultados em razão do aumento da salinidade. Portanto, uma vez que não são obtidas taxas de masculinização maiores em água leve ou moderadamente salinizada, a salinidade deve ser mantida próxima de 0 g L^{-1} para o protocolo de masculinização de larvas de tilápia-do-nilo em BFT, para melhor sobrevivência e maior biomassa dos peixes, assim como menor desperdício do alimento oferecido.

Termos para indexação: *Oreochromis niloticus*, BFT, inversão sexual, cloreto de sódio.

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Introduction

The biofloc technology (BFT) is an aquaculture system based on flocs composed of bacteria, microalgae, feces, decomposing organic matter, protozoa, cyanobacteria, small metazoan larvae, invertebrates, and other microorganisms (Ekasari et al., 2014; Ahmad et al., 2017). They are a source of nutrients, such as protein, lipids, vitamins, and minerals, showing probiotic effects (Wang et al., 2015; Khanjani & Sharifinia, 2020) and, therefore, they are beneficial for cultivated species (Silva et al., 2018). The use of this technology has been investigated for several species of fish, among which *Oreochromis niloticus* (Luo et al., 2014; Long et al., 2015; Ekasari et al., 2019).

A salinized BFT, obtained by the incorporation of sodium chloride (NaCl) in the water, can be viable, since BFT is an intensive system with limited water exchange (Alvarenga et al., 2018). The growth of Nile tilapia has been observed to be better in a slightly and moderately saline water (from 1 to 10 g L^{-1}) (Fridman et al., 2012; Alvarenga et al., 2018; Dawood et al., 2022). In addition, water salinity may increase the fish survival during eventual nitrite peaks, in comparison with nonsalinized systems, due to the competition between the chloride ion and nitrite for the same binding sites in gills (Tomasso, 2012). According to Alvarenga et al. (2018) salinized water, especially at about 6 g L^{-1}, is recommended in BFT to improve the growth performance of Nile tilapia in the initial grow-out phase – tilapias with 5 to 15 g –, after the masculinization period.

However, to the best of our knowledge, the best salinity for the masculinization period of Nile tilapia in BFT was not evaluated. This is the time when larvae weighs about 10 mg of body weight (under 11 mm length or at 10 days post-fertilization age) and are raised until 300–1,000 mg of body weight (Silva et al., 2022) and fed ration enriched with 17 a-methyltestosterone androgen during 28 days (Joshi et al., 2019). The masculinization of Nile tilapia larvae has been performed in different production systems, like ponds, recirculating aquaculture systems, and small tanks in BFT using freshwater (Homklin et al., 2012; David-Ruales et al., 2019; Silva et al., 2022). The use of slightly and moderately saline water for this phase of Nile tilapia larviculture in other production systems than BFT is not so common, but it seems to not interfere with the growth and the masculinization rate in brackish water (Moreira et al., 2011).

The pond system − probably the most common system in the world used to perform the masculinization process of Nile tilapias (Homklin et al., 2012) − is characterized by large cultivation structures and volumes of water stored and it does not allow of the water salinization, unless brackish water is used. However, the BFT system is based on smaller tanks, higher stocking densities, and greater control of water effluent release and water reuse and it can practice the use of salinization, if advantageous, routinely (Alvarenga et al., 2018). Thus, we posed the following questions: could slightly or moderately saline water improve the growth, survival, and the consume of the feed offered to Nile tilapia larvae raised in BFT, as it can for larger Nile tilapias (juveniles)? If feed consumption is improved and, consequently, the hormone too, could higher masculinization rates be obtained?

Therefore, the objective of this work was to evaluate the effect of different water salinity levels on the growth performance, survival, and masculinization rate of Nile tilapia larvae in a biofloc technology system.

Materials and Methods

The experiment was carried out in a greenhouse at aquaculture laboratory, in the Escola de Veterinária, of the Universidade Federal de Minas Gerais (UFMG), in the municipality of Belo Horizonte, in the state of Minas Gerais, Brazil. All procedures were approved by the Ethics Committee on the Use of Animals (CEUA- UFMG), protocol no. 312/2019.

A completely randomized design with seven treatments and three replicates was used, totaling 21 experimental units (150 L tank). Seven distinct levels of sodium chloride salinity (0, 2, 4, 6, 8, 10, and 12 g L^{-1}) were established in the culture water of tilapia larvae (*O. niloticus*), during the masculinization period (28 days of experiment after the yolk sac absorption), in a previously matured biofloc environment. To reach salinity levels above 2 g L^{-1}, an adaptation protocol was performed (Lemarié et al., 2004), and the transition of salinity was done gradually at 2 g L^{-1} per day, to avoid mortality, starting on the first day of the experiment. Therefore, the salinities at 2, 4, 6, 8, 10, and 12 g L^{-1} were achieved in the first, second, third, fourth, fifth, and sixth days of experimentation, respectively. Each experimental unit was equipped with a thermostat set at 28°C, and air stones and hoses were coupled to an air blower for constant aeration and water revolving.

A total of 6,300 larvae in the period after the yolk sac absorption − about 4 days of age after hatching, or about 8 days of age post fertilization − were randomly collected in the same day in pool of 10 hatched spawnings from females of a Chitralada line broodstock of the Nucleus of Studies in Nutrition, Genetics, and Technology in Aquaculture (NGTAqua) of the UFMG. They were randomly allocated in each tank of the experiment at a density of 2 larvae L^{-1} (300 individuals per tank). The large number of hatched spawnings from a broodstock of great genetic variability (with an inbreeding rate of less than 1.3%) − used to make the larvae pool − was important to avoid a family × treatment interaction effect.

During the 28 days of experiment, the larvae were fed a commercial powder ration (55% crude protein (Propescado-Nutriave Foods, Viana, ES, Brazil) enriched with the masculinizing hormone 17α-methyltestosterone (60 mg kg^{-1} of feed) (Silva et al., 2022). The ration offered per week was corrected, starting with a daily treat of 30% of the expected biomass, decreasing to 25, 20, and 15% in the subsequent weeks (Daudpota et al., 2016). The expected weight gain per week based on previous studies (Silva et al., 2022) and the expected number of live larvae were used to calculate the expected biomass for the following week. The total number of larvae that died during the week was deducted from the number of live animals in each tank for the determination of the expected live larvae per week. Feeding frequency was eight times a day (every hour from 8:00 h to 16:00 h).

At the 29^{th} day, one day after the end of the experiment, 60 randomly selected fingerlings from each tank were weighed and euthanized by eugenol overdose (300 mg L^{-1}); soon after, the gonad analysis was made through the aceto-carmine squash process (Silva et al., 2022), to determine the masculinization rate. This method consists of making an opening in the coelomic cavity, removing the viscera, and locating the gonads; then, the gonads are removed, fixed in Bouin liquid, and placed on a glass slide. For the procedures, we added a few drops of aceto-carmine (0.5 g aceto-carmine in 100 mL of 45% acetic acid) and squashed the tissue with a cover slip. The material was examined under a microscope using 100X magnification.

At the end of the experiment, the following performance variables were evaluated: amount of feed offered, final biomass, survival rate, average final body weight, and the quantity of feed offered as a proportion of final biomass. Feed consumption was not measured because it was not possible to collect leftovers of powder ration. All responses were obtained per experimental unit (tank). The final body weight was the average weight of the randomly selected fingerlings used for sexing in each tank. Biomass was determined by the average final body weight multiplied by the number of fingerlings alive at the end of experiment. Feed offered as a proportion of the final biomass (FOB) was the amount of feed supplied during the 28 days of experiment divided by the final biomass produced in each tank. Survival rate was calculated by the number of living animals, after the end of the experiment, divided by 300 (initial number), multiplying the result by 100. For the estimation of FOB, the final biomass was considered the gain of biomass, during the experiment, per tank, since the initial biomass is insignificant because it is a function of the negligible, average initial body weight after the absorption of yolk sac of Nile tilapia larvae (around 10 mg) that was the same for all tanks (larvae came from the same pool).

Dissolved oxygen, temperature, pH, and salinity were daily monitored using a multiparameter AKSO probe (Akso, São Leopoldo, RS, Brazil). Spectrophotometer readings of total ammonia nitrogen (TAN) were taken three times a week (Alvarenga et al., 2018). The calculation of nonionized ammonia (toxic ammonia) was performed considering the pH and the water temperature. Nitric nitrogen ($N\text{-}NO^{-}_2$) was quantified twice a week, and nitrate concentrations and alkalinity were measured twice, at the beginning and at the end of the experiment (Alves et al., 2017). Settleable solids were evaluated five times by the Imhoff cone method (Manduca et al., 2020) throughout the experiment. Salinity was kept with common salt (NaCl, 99%) in salinized treatments. Total suspended solids were assessed weekly (Alves et al., 2017; Manduca et al., 2020). All water quality variables were measured in all tanks.

When the TAN concentration reached values higher than 1 mg L^{-1}, cane sugar was added, at a ratio of 6 g carbon for each 1 g TAN (Manduca et al., 2021). Since the settleable solids level was satisfactory, no drainage was required (Manduca et al., 2020).

The data were analyzed with the aid of the R software (R Core Team, 2022). Nitrite data were transformed into natural logarithm to properly normalize the values. Linear regressions were fitted, and the residuals of the models were checked by the Shapiro-Wilk's normality test and the Levene's test of homoscedasticity, at 5% probability.

Results and Discussion

Water quality parameters were within the recommended range (Benli & Köksal, 2005; Alves et al., 2017; Martins et al., 2017; Monsees et al., 2017; Alvarenga et al., 2018; Nivelle et al., 2019; Manduca et al., 2020) for the development of Nile tilapia (Table 1), and there were no differences between treatments for temperature, dissolved oxygen, settleable solids, nitrate, total suspended solids, TAN, and toxic ammonia. In fact, the average TAN values were above 1 mg L^{-1} (6 g L^{-1} of salinity was the exception); however, toxic ammonia for all treatments showed means below the tolerated threshold (Table 1) recommended (Benli & Köksal, 2005).

The average pH followed a quadratic regression for salinities with a minimum at 2 g L^{-1}. There was a decrease of pH values in all treatments until the eighteenth day of the experiment (Figure 1). A pH increase was observed from day 18 to day 21, when it decreased again until the end of the experiment in all treatments. This reduction of pH in BFT is common and occurs due to the demand for carbonate and bicarbonate ions by microbiota subjected to BFT, which leads to a consumption of calcium carbonate and a reduction of pH levels (Avnimelech, 1999; Alves et al., 2017; Alvarenga et al., 2018). However, $CaCO_3$ concentration remained above 50 mg L^{-1}, according to the recommendations by Martins et al. (2017), and there was no need to supply the system with an inorganic carbon source during the experiment. Besides, the alkalinity levels also increased, and higher salinity levels were reached in accordance with average pH values (Table 1).

The accumulation of nitrite due to nitrification can be observed in BFT (Alves et al., 2017; Luo et al., 2020). Despite the mature biofloc used to fill the tanks, the nitrite concentration increased at higher salinities (Table 1). Higher peaks of nitrite (Figure 2) were observed for 6 and 8 g L^{-1}, at the middle of the masculinization period (14th day), and for 8 (a second

Table 1. Means, coefficient of variation (CV), and linear regression models of water quality variables of masculinization of Nile tilapia (*Oreochromis niloticus*) larvae reared at different salinities in biofloc technology.

Reference values for tilapia culture	Salinity (g L^{-1})							CV (%)	Regression models; R^2	p-value(1)
	0	2	4	6	8	10	12			
Temperature (°C)(2)	27.17	27.03	26.97	27.01	27.13	27.34	27.51	1.16	-	0.0697ns
DO (mg L^{-1})(3)	7.16	7.37	7.44	7.46	7.41	7.32	7.11	2.58	-	0.6420ns
pH(4)	8.06	7.76	7.86	8.02	7.95	8.16	8.27	1.3	$Y = 7.98 - 0.048x + 0.006x^2$; R^2=0.75	0.06 and 0.005
$CaCO_3$ (mg $CaCO_3$ L^{-1})(5)	82.5	75.83	87.5	90.83	102.5	98.33	106.67	13.0	$Y = 77.83 + 2.37x$; R^2=0.85	0.0009
SS (mL L^{-1})(6)	8.37	4.61	5.45	7.83	7.09	6.27	7.64	41.24	-	0.7435ns
TSS (mg L^{-1})(7)	202.92	216.25	267.92	243.33	218.33	210.42	203.33	16.15	-	0.6157ns
TAN (mg L^{-1})(8)	1.05	1.31	1.64	0.85	1.42	1.53	1.45	21.33	-	0.1957ns
ToxTAN (µg L^{-1})(9)	37.98	46.90	60.22	31.18	53.96	58.14	56.14	22.12	-	0.0955ns
Nitrite (mg L^{-1})(10)	0.46	1.17	0.89	1.19	5.86	3.04	1.94	100.36	$Y = -0.50 + 0.13x$(11); R^2=0.71	0.0008
$NO_3^-{}_i$ (mg L^{-1})(12)	144.45	149.15	186.91	168.01	169.86	159.45	164.75	20.69	-	0.5140ns
$NO_3^-{}_f$ (mg L^{-1})(13)	272.16	305.46	285.80	219.51	261.90	239.09	241.83	14.14	-	0.23ns
Salinity (g L^{-1})	0.22	2.06	4.02	5.9	7.72	9.46	11.61	1.13	$Y = 0.21 + 0.94x$; R^2=0.99	<0.0001

(1)Linear regression was not significant (ns), in accordance with the analysis of variance, at 5% probability. (2)27–32°C (Nivelle et al., 2019). (3)DO (dissolved oxygen) > 4 mg L^{-1} (Alves et al., 2017). (4)pH = 6–9 (Alvarenga et al., 2018). (5)$CaCO_3$ (alkalinity) > 50 mg $CaCO_3$ L^{-1} (Martins et al., 2017). (6)SS (settleable solids) < 100 mL L^{-1} (Manduca et al., 2020). (7)TSS (total suspended solid) < 1,000 mg L^{-1} (Manduca et al., 2020). (8)TAN (total ammonia nitrogen) < 1 mg L^{-1} (Alvarenga et al., 2018). (9)ToxTAN (toxic ammonia) < 1 mg L^{-1} (Benli & Köksal, 2005). (10)Nitrite < 8 mg L^{-1} (Alvarenga et al., 2018). (11)Nitrite data were transformed in natural logarithm to properly normalize the values and, then, a linear regression model was fitted. (12)$NO_3^-{}_i$ (initial nitrate). (13)$NO_3^-{}_f$ (final nitrate) < 500 mg L^{-1} (Monsees et al., 2017).

peak), 10, and 12 g L^{-1} at the middle of the last week of the experimental period. The average values of nitrate of the final collection were different ($p<0.05$) from the first ones, which shows that there was a nitrate increase due to the nitrite produced by nitrifyng bacteria, as observed by Manduca et al. (2020); however, the nitrate

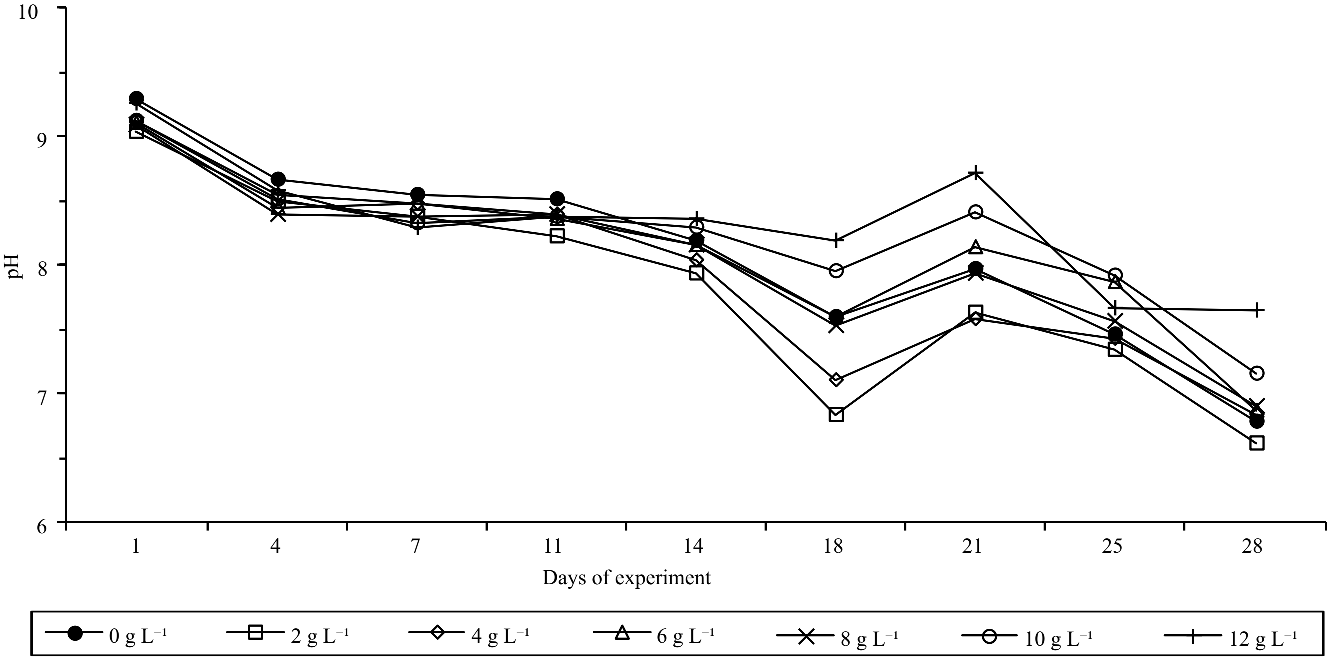

Figure 1. Means of pH of seven different salinity levels (0, 2, 4, 6, 8, 10, and 12 g L^{-1}) from tanks with biofloc, throughout the masculinization experiment of Nile tilapia (*Oreochromis niloticus*) larvae.

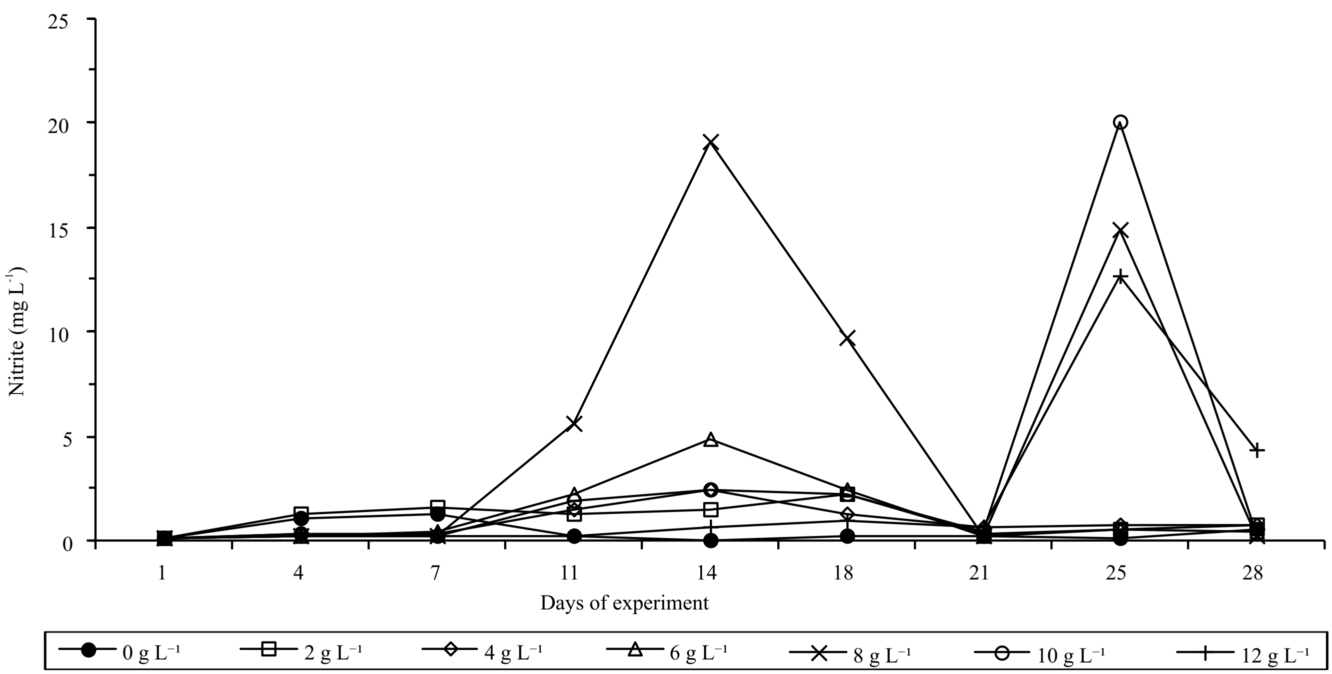

Figure 2. Nitrite concentrations of seven different salinity levels (0, 2, 4, 6, 8, 10, and 12 g L^{-1}) from tanks with biofloc, throughout the masculinization experiment of Nile tilapia (*Oreochromis niloticus*) larvae.

concentration was similar among treatments at the end of the experiment. The results of pH, alkalinity, nitrite, and nitrate indicate that the increase of salinity inhibits the activity and/or growth of bacteria. These results corroborate the findings by Alvarenga et al. (2018), who have suggested that the increase of water salinity promotes the reduction of bacterial activity, mainly the activity of nitrifiers, causing higher peaks of nitrite and the average increase of this nitrogenous compound in water. Bacteria that grew in BFT were possibly adapted to a low salinity, since an initial mature floc in freshwater was used.

In an experiment carried out in a salinized clear water system (Luz et al., 2013), the survival rates were not different between treatments at 0 and 2 g L^{-1}, with an average above 94%, and they were different between 4 g L^{-1} (43%) and 6 g L^{-1} (0%). It is important to note that the adaptation recommended by Lemarié et al. (2004) was not applied by Luz et al. (2013), which severely compromised the tilapia survival. A superior survival at lower salinities was also observed (Table 2) in the present study, since the high peaks of nitrite for higher salinity levels may have contributed to lower survival rate (minimal point at 8.57 g L^{-1}).

Studies have shown that Nile tilapia shows better performance and growth at moderate salinities of approximately 5 to 12 g L^{-1} (Fridman et al., 2012; Alvarenga et al., 2018), for growth phases after larviculture. In an experiment carried out in the same laboratory as the present study, Alvarenga et al. (2018) showed that animals from 0.5 to 20 g of live weight, that is, in a phase sequential to the larviculture phase, had a superior performance at salinity range from 4 to 8 g L^{-1}. In the present study, there were no significant effects of the salinity increase over the final body weight. However, the stocking density in individuals per cubic meter had a direct effect on the final average weight of the larvae, fingerlings, juveniles, and adult Nile tilapias (Manduca et al., 2021), favoring the weight gain of larvae in experimental units with a lower number of individuals per cubic meter. With lower survival at higher salinities, the final average weight in these treatments may have been the same as the other levels, due to the lower stocking density. Once the final weights were equal, salinity levels with higher survival rates would result in higher biomass, as observed in the present study (Table 2), which leads us to infer that younger animals in the larval phase do not tolerate salinities at which juveniles and adults present satisfactory performance for production, even adopting the adaptation period (Lemarié et al., 2004).

The reduction of feed offered in accordance with the increase of salinity occurred because a correction of feed offered was made following the mortality observed over the weeks. Despite the correction made for the amount of feed offered, during the first half of the experimental period (two weeks), the amount of feed was calculated for almost 300 larvae for all experimental units, once mortalities caused by nitrite peaks had not been observed yet. This fact was worse for the salinity treatments at 8, 10 and 12 g L^{-1}, which caused mortalities observed at the last days of the last week of the masculinization period, following the nitrite peaks, after the last feed offered correction. This way, the results of the amount of feed as a proportion of final biomass increased following the salinity levels.

Similar rates of masculinization were obtained, with no difference between treatments (Table 2). This result

Table 2. Means, coefficient of variation (CV), and linear regressions models of growth performance variables and masculinization rate (MR) of Nile tilapia (*Oreochromis niloticus*) larvae, during masculinization protocol at different salinity levels in biofloc technology.

Variable	Salinity (g L^{-1})							CV (%)	Regression models; R^2	p-value[(1)]
	0	2	4	6	8	10	12			
Offered feed (g)	206.67	179.83	181.97	120.97	145.17	114.13	118.43	20.87	$Y = 198.83 - 7.73x$; $R^2=0.81$	0.0002
Final biomass (g)[)]	219.95	194.13	174.36	88.21	88.13	72.34	62.16	27.8	$Y = 213.43 - 14.16x$; $R^2=0.88$	<0.0001
FOB	0.95	0.93	1.06	1.4	1.86	1.95	1.69	33.66	$Y = 0.86 + 0.09x$; $R^2=0.81$	0.015
Final body weight (g)	0.74	0.76	0.69	0.73	0.83	0.68	0.40	29.95	-	0.1107[ns]
Survival rate (%)	98.78	84.44	84.44	41.33	46.67	32.67	67.11	33.79	$Y = 106.5 - 13.89x + 0.81x^2$; $R^2=0.75$	0.006; 0.04
MR (%)	96.12	93.86	92.47	96.60	86.82	92.51	86.61	6.75	-	0.0595[ns]

[(1)]Linear regression was not significant, in accordance with the analysis of variance, at 5% probability. FOB, feed offered as a proportion of final biomass.

was expected, since there is no evidence that salinity affects masculinization in Nile tilapia (Moreira et al., 2011). However, this verification is important, as differences for growth performance can indirectly alter the masculinization rates.

Conclusions

1. The use of salinized water during the masculinization period of Nile tilapia (*Oreochromis niloticus*) larvae should not be recommended, when the biofloc technology system is applied, since higher masculinization rates are not obtained at slight and moderate levels of saline water.

2. Water salinity close to 0 g L^{-1} promotes higher biomass and survival rates, and lower waste of the offered feed.

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