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Scientific Article

Larviculture of Nile tilapia (*Oreochromis niloticus*) in biofloc and clear water systems: masculinization with 17 α-methyltestosterone

Ugo Lima Silva[1]*, Plinio Gomes Júnior[1], Dario Rocha Falcon[1], Nivaldo Ferreira do Nascimento[1], Renata Akemi Shinozaki-Mendes[1]

[1]Universidade Federal Rural de Pernambuco – Serra Talhada (PE), Brazil.

***Corresponding author:** ugo.silva@ufrpe.br

ABSTRACT

We evaluated the use of the hormone 17α-methyltestosterone during the larviculture of Nile tilapia in biofloc and clear water systems. A completely randomized experimental design was adopted with four treatments and four replications: biofloc system without dietary hormone incorporation (BFT-D); biofloc system with dietary hormone incorporation (BFT-H); clear water without hormone incorporation in the diet (CLW-D); and clear water with hormone incorporation in the diet (CLW-H). The 28-day larviculture of Nile tilapia was carried out using 16 tanks with capacity of 15 L. The evaluations included water parameters, growth, survival, and gonad histology. The biofloc system displayed higher values for alkalinity, total suspended solids, settling solids, and turbidity compared to the clear water system ($p < 0.05$). Nevertheless, no difference ($p > 0.05$) was observed for in final weight, weight gain, feed conversion factor, survival, and growth rate between treatments. Then, tilapia post-larvae can be successfully reared in both biofloc and clear water systems without any negative impact on their zootechnical performance. Nonetheless, such results showed that tilapia can be reared in alternative systems, which can increase the production of this species.

Keywords: Closed systems; Fish farming; Zootechnical performance; Histology; Water quality.

Larvicultura de tilápia-do-nilo (*Oreochromis niloticus*) em sistemas de bioflocos e águas claras: masculinização com 17 α-metiltestosterona

Resumo

Foi avaliado o uso do hormônio 17α-metiltestosterona durante a larvicultura de tilápia-do-nilo em sistemas de bioflocos e águas claras. Adotou-se um delineamento experimental completamente casualizado com quatro tratamentos e quatro repetições: sistema de bioflocos sem incorporação de hormônio na dieta (BFT-D); sistema de bioflocos com incorporação de hormônio na dieta (BFT-H); água clara sem incorporação de hormônio na dieta (CLW-D); e água clara com incorporação de hormônio na dieta (CLW-H). A larvicultura foi realizada por 28 dias utilizando 16 caixas com capacidade de 15 L. A qualidade da água, o crescimento, a sobrevivência e a histologia gonadal foram avaliados. O sistema de bioflocos apresentou valores mais elevados para alcalinidade, sólidos suspensos totais, sólidos em suspensão sedimentáveis e turbidez ($p < 0,05$), no entanto não foi observada diferença ($p > 0,05$) no peso final, ganho de peso, fator de conversão alimentar, sobrevivência e taxa de crescimento entre os tratamentos. Desse modo, as pós-larvas de tilápia podem ser criadas com sucesso tanto em sistemas de bioflocos quanto em sistemas de água clara sem nenhum impacto negativo em seu desempenho zootécnico, o que pode aumentar a produção dessa espécie.

Palavras-chave: Sistemas fechados; Piscicultura; Desempenho zootécnico; Histologia; Qualidade da água.

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INTRODUCTION

Fish farming in Brazil has continued to grow, reaching 860,355 tons in 2022 (PEIXE BR, 2023). The main species produced is the Nile tilapia (*Oreochromis niloticus*), which represents 63.5% of all Brazilian fish farming and amounted to 534,005 tons in 2021, making Brazil the fourth largest producer of Nile tilapia in the world (FAO, 2022; PEIXE BR, 2023). This is largely due to the species' several advantages for fish farming, including fast growth, rusticity, resistance to poor water quality, tolerance to a wide temperature range, high prolificity, and ability to acclimate to high salinity concentrations (Khanjani et al., 2022; Valentin et al., 2015).

However, despite these advantageous qualities, there are still challenges in the breeding process, such as parental care, high reproductive capacity, and early sexual maturation, which can lead to overpopulation in cultivation nurseries, competition for space and food, and reduced growth rates of individuals (Mendes and Carvalho, 2016; Zanoni et al., 2013). As a result, the production of male-only populations in tilapia fish farming has become a common practice. Rearing only males has also added benefits such as increased growth, better flesh quality, and greater uniformity (Li et al., 2022; Wang and Shen, 2018).

Several methods for tilapia sex control have been described, including hybridization, chromosome manipulation (polyploidy, gynogenesis, and androgenesis) (Arai and Fujimoto, 2018; Chen et al., 2018), heat temperature (D'Cotta et al., 2001; Khater et al., 2017), and sexual masculinization using hormones (Asad et al., 2010; Popma and Lovshin, 1995). However, most farmers use 17 α-methyltestosterone (MT) hormones, primarily because the process is cheap, efficient, simple, and the hormone is easily excreted after treatment (Asad et al., 2010; Baroiller; D'Cotta, 2018; Bombardelli et al., 2007). Methods of MT administration include immersion and injection, but the most commonly used method is incorporating the hormone into the diet (Baroiller & D'Cotta, 2018).

Sex steroids play an important role in sexual differentiation in non-mammalian vertebrates (Nakamura, 2010). The hormone must be administrated before the sexual differentiation of gonads and its efficacy is generally assessed by gonad histology (Jensi et al., 2016; Solomon and Okomoda, 2012). However, the use of MT in traditional aquaculture systems results in the discharge of effluent into the environment, which is not environmentally friendly (Mlalila et al., 2015; Thanasupsin et al., 2021). To address this issue, closed systems such as recirculating aquaculture systems and biofloc technology (BFT) could be a more sustainable alternative to reduce or avoid the discharge of MT (Avnimelech, 2012; Ahmad et al., 2017; Khanjani et al., 2024). Closed systems also reduce water usage and can increase productivity while reducing costs (Avnimelech, 1999, 2012; De Schryver et al., 2008; Emerenciano et al., 2017).

In BFT, a heterotrophic microbial community is developed by manipulating the relationship between carbon (C) and nitrogen (N) through the addition of an external source of organic carbon (Khanjani et al., 2023). This leads to the formation of bioflocs, which are aggregates of bacteria, microorganisms, excreta, and feed, that are consumed by the fish (Avnimelech, 2007; Azim; Little, 2008; Crab et al., 2009; Crab et al., 2012). However, just few studies have been developed comparing the use of the 17α-MT hormone during larviculture of Nile tilapia in biofloc and clear water systems. This study aimed to evaluate the use of the hormone in these systems, focusing on water quality, zootechnical performance, and gonadal development.

MATERIALS AND METHODS

The experiment was conducted at the Laboratory for Experimentation of Aquatic Organisms located at the Universidade Federal Rural de Pernambuco's Academic Unit of Serra Talhada in Pernambuco, Brazil. The study received ethical approval from the Ethics Committee on the Use of Animals with the protocol number 23082.006461/2017-85.

Animals and facilities

The larviculture of tilapia was performed from March 30th to April 29th, 2019, a period of 28 days. The experimental setup consisted of 16 circular polyethylene tanks with the capacity of 15 L (0.05 m^2). The tanks using the biofloc technology molasses was applied as a carbon source, with no water renewal except to compensate for evaporation losses. In contrast, the tanks in the clear water system were periodically siphoned, and the water (drawn from an artesian well) was renewed daily. Each tank was continuously aerated using air stones connected to a radial compressor, and covered with screens to prevent the fish from jumping out.

Design and experimental procedures

A completely randomized experimental design was employed, consisting of four treatments and four replications:

- T1: biofloc system without the incorporation of hormones (BFT-D);
- T2: biofloc system with the incorporation of hormones in the diet (BFT-H);
- T3: clear water system without the incorporation of hormones (CLW-D);

- T4: clear water system with the incorporation of hormones in the diet (CLW-H).

The carbon:nitrogen (C:N) ratio was adjusted daily to 15:1 by adding a source of organic carbon (liquid molasses) to stimulate the growth of heterotrophic bacteria. This balanced ratio ensures an optimal environment for bacterial metabolism, in which carbon-rich organic compounds from the molasses provide energy and building blocks for cellular processes, while the available nitrogen supports protein synthesis and overall growth.

The quantities of molasses were calculated based on the required C:N ratios and the carbon content in the molasses (%C), according to Eq. 1 (Avnimelech, 1999):

$$\Delta_{Molasses} = [(Q_{Feed} \times \%N_{Feed} \times \%N_{Excretion}) \times (C:N)] \times \%C^{-1} \quad (1)$$

Where: Q_{Feed}: the amount of feed offered daily; $\%N_{Feed}$: the amount of nitrogen inserted into the system ($\%Protein \times 6.25^{-1}$); $\%N_{Excretion}$: the flux of ammonia in water directly from excretion or indirectly by microbial degradation of organic nitrogen residues.

The molasses used contains about 30% carbon in relation to dry matter. Therefore, using feed containing 38% protein (6.08% N) and considering that 50% of the nitrogen in the feed is excreted, there is Eq. 2:

$$(\%N_{Excretion}): \Delta_{Molasses} = [(Q_{Feed} \times 0.0608 \times 0.5) \times (15)] \times 0.30^{-1} \quad (2)$$

Biological material and food management

Newly hatched *O. niloticus* larvae were obtained from a commercial company and randomly and equally distributed among the tanks, with a stocking density of 8 larvae L^{-1} (120 larvae/tank). The larvae were fed a powdered diet containing 38% digestible protein on a daily basis (Table 1). The experimental diet was formulated and balanced based on the digestible protein values as determined by Cowey (1985), and the other ingredients were selected according to the food digestibility coefficients as established by Pezzato et al. (2002).

The masculinizing hormone 17α-MT was incorporated in the diet following Popma and Green (1990). The hormone (60 mg) was dissolved in 400 mL of absolute alcohol and then mixed with the fish diet (1 kg). The feed was stocked at room temperature (in dark conditions) for alcohol evaporation. Afterwards, the feed was stored in plastic containers and were stored in a refrigerator at -20°C. Fish were fed six times a day (7:30 a.m., 9:30 a.m., 11:30 a.m., 1:30 p.m., 3:30 p.m., and 5:30 p.m.) at a ratio that allowed maximum intake without losses.

Table 1. Formulation and balancing of the experimental diet used during the larviculture of the Nile tilapia *Oreochromis niloticus*.

Feed ingredients	**%**
Soybean meal	20.64
Fishmeal	26.57
Gut meal	25.09
Corn grain	17.58
Wheat bran	1.91
Soy oil	7.23
L-Tryptophan	0.10
L-Threonine	0.26
Butylated hydroxytulene	0.02
Premix vitamin and mineral*	0.50
NaCl	0.10
Total	100
	Nutritional value
Dry matter (%)	98.01
Gross energy (kg/kcal)	3674.43
Crude protein (%)	44.04
Digestible protein (%)	38.60
Crude fiber (%)	2.11
Ether extract (%)	16.52

*Additives: sodium (Min) 185 mg; vitamin A (Min) 20,000 IU; vitamin D3 (Min) 5600 IU; vitamin E (Min) 400 IU; vitamin K3 (Min) 37 mg; vitamin C (Min) 2,000 mg; thiamine (B1) (Min) 40 mg; riboflavin (B2) (Min) 40 mg; folic acid (Min) 12.5 mg; biotin (Min) 0.13 mg; niacin (Min) 212 mg; calcium pantothenate (Min) 100 mg; copper (Min) 19.5 mg; iron (Min) 146 mg; manganese (Min) 58 mg; selenium (Min) 0.95 mg; zinc (Min) 129 mg; mannan-oligosaccharide (Min) 60 mg; *Pediococcus acidilactici* 1.5×10^9 CFU.

Water quality

Water samples were collected weekly for analysis of total ammonia nitrogen (mg/L), nitrite (mg/L), nitrate (mg/L), inorganic phosphate (mg/L), alkalinity (mg/L), turbidity, settleable solids (mL/L), and total suspended solids (g/L). Total ammonia nitrogen, nitrite, nitrate, inorganic phosphate, and alkalinity were measured using a photometer (YSI 9500). Sedimented solids (SS) and turbidity were measured using an Imhoff settling cone and a turbidimeter, respectively. Total suspended solids analysis allowed for examination of organic fractions (fixed solids) and inorganic fractions (volatile solids), following American Public Health Organization guidelines (Eaton et al., 1995).

Fish performance assessment

At the beginning and the end of the experiment, fish weight (d = 0.001 g) and total length (0.01 mm) were measured. At 28 days, survival (%) and feed conversion factor were also calculated.

Histological evaluation of the gonads

For histology evaluation, samples were collected at two, 14, and 28 days. In each time, 10 fishes for treatment were randomly collected (total of 120 specimens). The fish were anesthetized in eugenol solution and fixed in 4% buffered formalin solution for 24 hours. Afterwards, they were stored in 70% alcohol and processed for routine histology histology in increasing alcohol series, diaphanized in xylene and impregned and embedded in paraffin at 60°C (adapted from Junqueira and Junqueira, 1983) for tissue differentiation. Tissue blocks were sectioned at 5 µm and stained with hematoxylin-eosin method. Sex was identified under optical microscope by the presence of oocytes (female) or seminiferous tubules (male). When these features were not present and sex were not identified, the individual was considered undifferentiated.

Statistical analyses

The data was tested for normality (using the Shapiro-Wilk's test) and homoscedasticity (using the Bartlett's test). The variables of water quality and fish performance were analyzed using analysis of variance, followed by the Tukey's test. To compare the proportion of individuals with undetermined sex, males, and females in each treatment, the parametric test of proportion was used. Significance was set at 0.05.

RESULTS

Monitoring of water quality

Table 2 displays the daily water quality variables monitored in both the biofloc and clear water systems. There were no significant differences between the treatments in terms of temperature, dissolved oxygen, and pH ($p > 0.05$). However, the biofloc system showed higher values for conductivity, total dissolved solids, and salinity ($p < 0.05$).

Table 2. Mean values ± standard deviation (minimum–maximum in parentheses) of the physicochemical variables of water quality monitored daily in the tilapia *Oreochromis niloticus* larviculture using masculinizing hormone in biofloc and clear water breeding systems.

Variables	Experimental treatments				p-value
	BFT-D	BFT-H	CLW-D	CLW-H	
Temperature (°C)	25.10 ± 0.10 (22.50–28.10)	25.20 ± 0.20 (22.60–28.90)	25.30 ± 0.30 (22.60–28.70)	25.30 ± 0.20 (22.50–28.80)	0.3036
Dissolved oxygen ($mg \cdot L^{-1}$)	6.20 ± 0.00 (3.20–9.98)	6.1 ± 0.10 (3.10–10.20)	6.20 ± 0.30 (3.00–10.23)	5.30 ± 0.10 (3.20–10.10)	0.2221
Electrical conductivity ($mS \cdot cm^{-1}$)	3.00^{a} ± 0.54 (1.95–3.52)	2.99^{a} ± 0.37 (2.09–3.50)	2.18^{b} ± 0.12 (2.01–2.47)	2.17^{b} ± 0.05 (1.98–2.80)	0.0001
Total dissolved solids ($g \cdot L^{-1}$)	1.96^{a} ± 0.26 (1.37–2.20)	1.94^{a} ± 0.12 (1.11–2.14)	1.41^{b} ± 0.06 (1.28–1.86)	1.41^{b} ± 0.12 (1.32–1.86)	0.0001
Salinity ($g \cdot L^{-1}$)	1.60^{a} ± 0.00 (1.00–1.93)	1.60^{a} ± 0.00 (1.00–1.72)	1.00^{b} ± 0.00 0.98–1.26	1.10^{b} ± 0.00 (1.00–1.44)	0.0001
pH	7.60 ± 0.00 (7.30–7.98)	7.60 ± 0.00 (7.20–7.84)	7.70 ± 0.00 (7.20–7.98)	7.70 ± 0.00 (7.10–7.95)	0.4032

BFT-D: hormone-free biofloc system; BFT-H: biofloc system with hormone; CLW-D: hormone-free clear water system; CLW-H: clear water system with hormone; Absence of superscript letters between columns do not differ significantly between treatments ($p > 0.05$).

Table 3 shows the weekly water quality variables monitored in the biofloc and clear water cultivation systems. The biofloc system displayed higher values for alkalinity, total suspended solids, settling solids, and turbidity compared to the clear water system ($p < 0.05$).

Zootechnical performance

No difference ($p > 0.05$) was observed for in final weight, weight gain, feed conversion factor, survival, and growth rate between treatments (Table 4).

Table 3. Mean values ± standard deviation (minimum–maximum in parentheses) of the physicochemical variables of water quality monitored weekly in the tilapia *Oreochromis niloticus* larviculture using masculinizing hormone in biofloc and clear water breeding systems*.

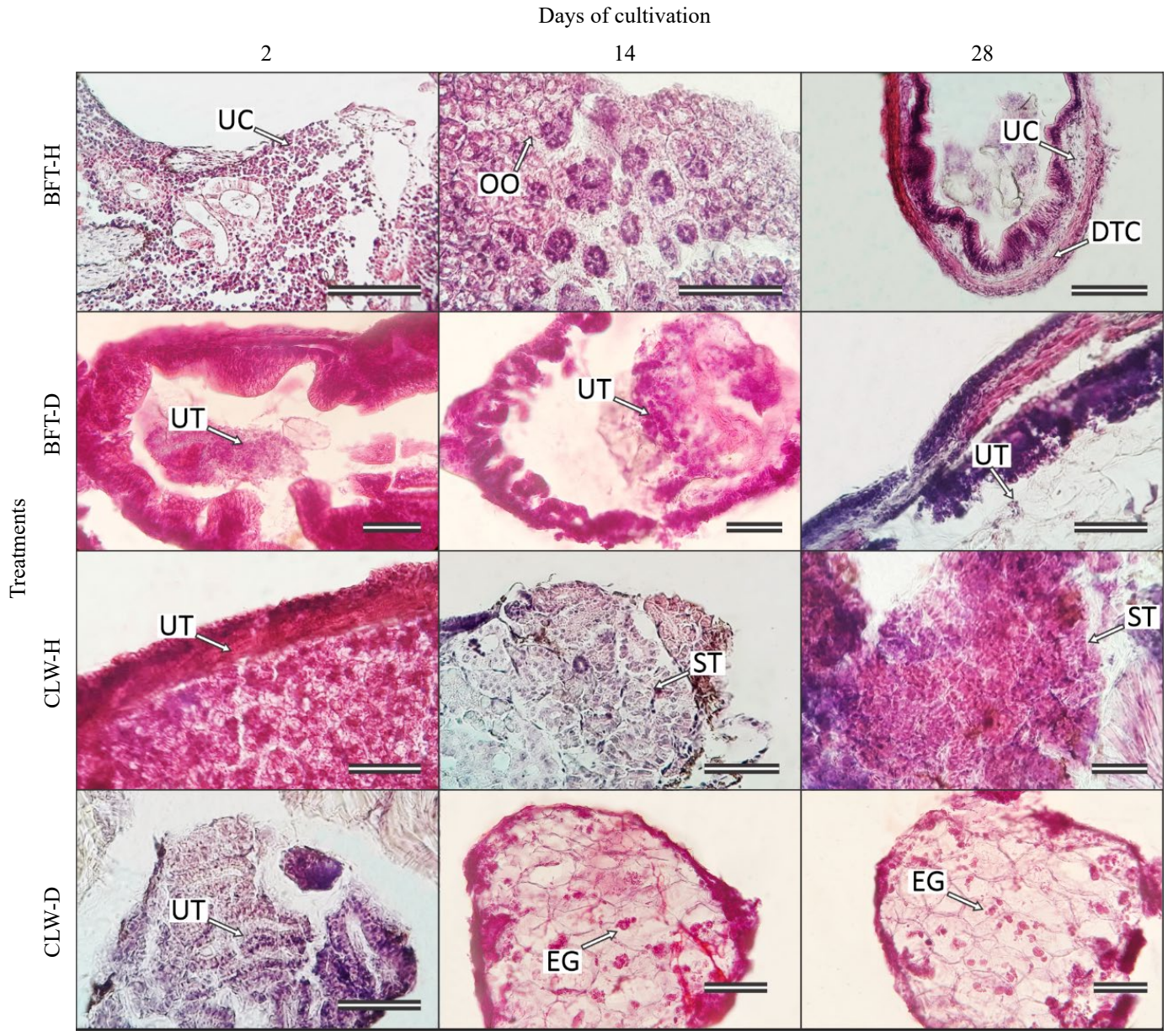
Variables	Experimental treatments				p-value
	BFT-D	BFT-H	CLW-D	CLW-H	
Total ammoniacal nitrogen ($mg \cdot L^{-1}$)	$0.19^{a} \pm 0.37$ (0.02–1.01)	$0.22^{a} \pm 0.38$ (0.01–1.06)	$0.15^{a} \pm 0.39$ (0.01–1.01)	$0.09^{a} \pm 0.40$ (0.01–1.01)	0.1720
Nitrite ($mg \cdot L^{-1}$)	$0.27^{a} \pm 1.48$ (0.00–5.00)	$0.23^{a} \pm 1.63$ (0.00–6.70)	$0.91^{a} \pm 1.88$ (0.00–10.00)	$0.29^{a} \pm 2.99$ (0.00–12.50)	0.6032
Nitrate ($mg \cdot L^{-1}$)	$60.17^{a} \pm 102.31$ (0.18–352.00)	$57.72^{a} \pm 112.38$ (0.24–300.00)	$57.82^{a} \pm 76.67$ (0.22–244.00)	$59.50^{a} \pm 67.34$ (0.17–232.00)	0.9674
inorganic phosphate ($mg \cdot L^{-1}$)	$29.70^{a} \pm 6.13$ (16.60–55.50)	$31.05^{a} \pm 4.99$ (20.90–42.30)	$26.27^{a} \pm 4.53$ (13.50–31.10)	$24.27^{a} \pm 5.12$ (13.70–34.80)	0.2265
Total alkalinity ($mg \cdot L^{-1}$)	$256.25^{a} \pm 51.32$ (160.00–320.00)	$262.50^{a} \pm 43.90$ (160.00–420.00)	$220.00^{b} \pm 27.10$ (155.00–310.00)	$197.50^{b} \pm 24.34$ (145.00–260.00)	0.0002
Total suspended solids ($g \cdot L^{-1}$)	$0.75^{a} \pm 1.22$ (0.20 – 4.39)	$0.75^{a} \pm 0.72$ (0.16–3.12)	$0.16^{b} \pm 0.24$ (0.11–1.16)	$0.19^{b} \pm 0.18$ (0.11–0.75)	0.0001
Settling solids ($mL \cdot L^{-1}$)	$39.75^{a} \pm 13.63$ (16.00–70.00)	$34.62^{a} \pm 10.80$ (17.00–60.00)	$0.00^{b} \pm 0.00$ (0.00–0.00)	$0.00^{b} \pm 0.00$ (0.00–0.00)	0.0001
Turbidity (NTU)	$137.12^{a} \pm 82.83$ (3.84–256.10)	$128.32^{a} \pm 94.94$ (3.12–386.52)	$5.77^{b} \pm 4.76$ (0.84–20.90)	$6.73^{b} \pm 5.05$ (0.84–25.80)	0.0001

BFT-D: hormone-free biofloc system; BFT-H: biofloc system with hormone; CLW-D: hormone-free clear water system; CLW-H: clear water system with hormone; Different letters in the same lines differ statistically by Tukey's test ($p < 0.05$).

Table 4. Zootechnical performance of *Oreochromis niloticus* tilapia larvae using masculinizing hormone in biofloc and clear water breeding systems.

Variables	Experimental treatments				p-value
	BFT-D	BFT-H	CLW-D	CLW-H	
Final weight (g)	0.10 ± 0.02	0.13 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.0576
Final length (mm)	17.8 ± 1.5	17.2 ± 2.1	17.0 ± 1.1	17.5 ± 1.0	0.1492
Biomass gain (g)	8.11 ± 1.03	10.48 ± 0.76	7.66 ± 1.82	7.76 ± 0.69	0.1365
Total feed supplied/biomass gain	1.97 ± 0.24	1.46 ± 0.11	2.16 ± 0.50	2.02 ± 0.17	0.1725
Survival (%)	66.67 ± 7.08	68.13 ± 1.83	67.29 ± 11.83	66.46 ± 2.83	0.9965
TCE (% day^{-1})	0.17 ± 0.01	0.17 ± 0.00	0.16 ± 0.00	0.16 ± 0.00	0.0650

BFT-D: hormone-free biofloc system; BFT-H: biofloc system with hormone; CLW-D: hormone-free clear water system; CLW-H: clear water system with hormone; Absence of letters on a same line do not differ statistically by analysis of variance ($p > 0.05$); TCE = 100 (Ln final weight - Ln initial weight)/breeding duration.

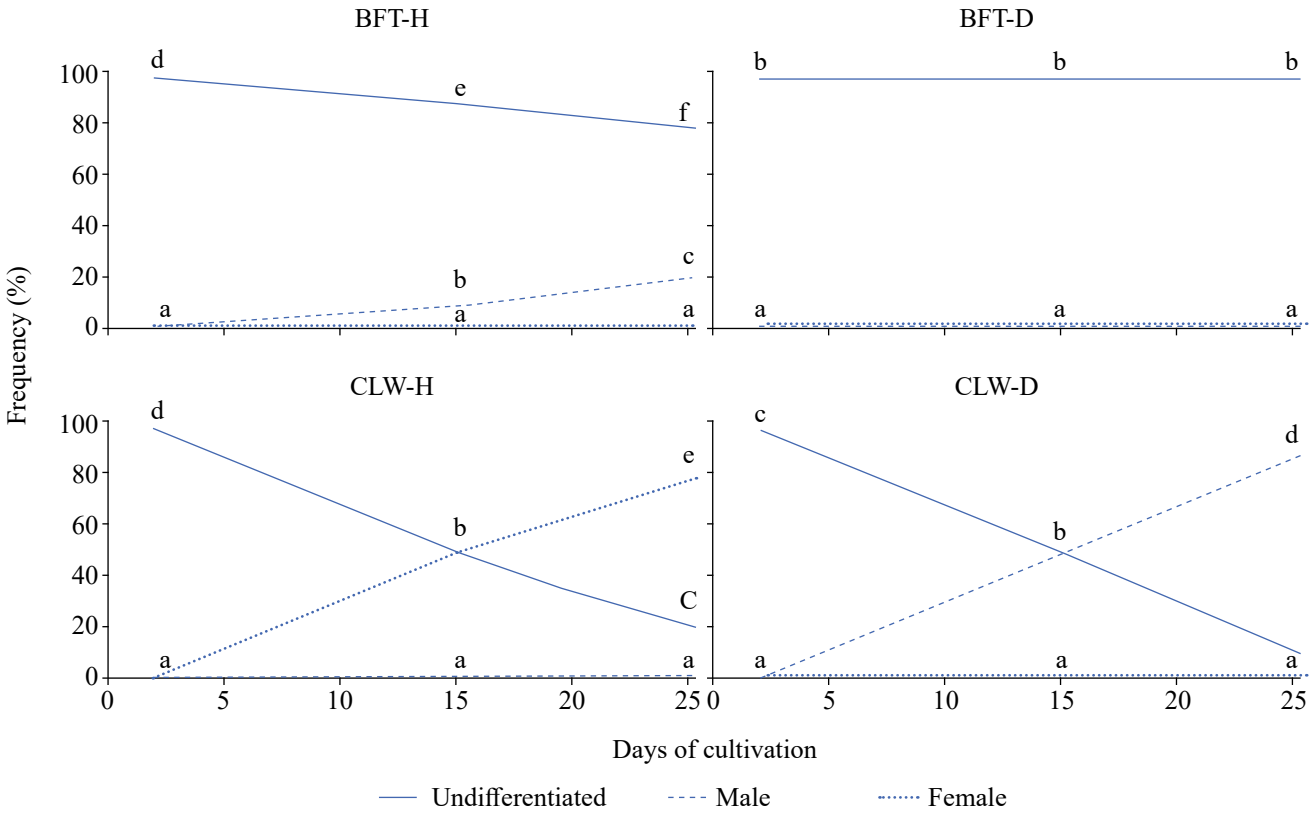


BFT-D: hormone-free biofloc system; BFT-H: biofloc system with hormone; CLW-D: hormone-free clear water system; CLW-H: clear water system with hormone. UC: undifferentiated cells; DTC: dense connective tissue; UT: undifferentiated tissue; EG: early gametogenesis; OO: oocytes; ST: seminiferous tubules.

Figure 1. Photomicrograph of *Oreochromis niloticus* tilapia gonads cultivated using masculinizing hormone in biofloc and clear water breeding systems. Scale bar: 80 µm.

Gonadal histology

Histological analysis showed that all fishes at two days had undifferentiated gonads with a large amount of germ cells and dense fibrous connective tissue (Fig. 1). At 14 days, in some fish, the presence of seminiferous tubules, initial gametogenesis, and oocytes in development were possible to verify. At 28 days, in the CLW-H treatment it was possible to identify the formation of 80% of male gonads and no females. In the CLW-D treatment, some gonads were in the initial gametogenesis and others remained undifferentiated. They presented an abundance of dense fibrous connective tissue and undifferentiated cells, with a tendency to form 90% females and no males (Figs. 1 and 2).



BFT-D: hormone-free biofloc system; BFT-H: biofloc system with hormone; CLW-D: hormone-free clear water system; CLW-H: clear water system with hormone

Figure 2. Comparison of the proportion of individuals with undetermined sex, males, and females over time in each treatment. Different letters indicate statistical difference by the Z test ($p < 0.05$).

Most fish in the BFT-H treatment showed undifferentiated characteristics, few females, and no males, while in the BFT-D treatment fish remained undifferentiated during the study period.

DISCUSSION

Water quality

The physical and chemical parameters of water quality monitored daily showed no significant differences among the treatments and remained within acceptable limits for the species (Emerenciano et al., 2017; Furuya and Barros, 2012). However, the only exception was temperature. According to Emerenciano et al. (2017), the ideal temperature range for Nile tilapia is 28–30°C, while Furuya and Barros (2012) reported that tilapia can develop well at 25–31°C. The minimum and maximum water temperatures recorded were 22.5 and 28.9°C, with an average of 25°C. This low temperature can likely be attributed to the rainy season and decreased exposure to sunlight in the tank structures.

Emerenciano et al. (2017) stated that the minimum concentration of dissolved oxygen required for tilapia in a biofloc system is 4 mg/L. In this study, the minimum value recorded was 3.1 mg/L, and the maximum was 10.2 mg/L, with fluctuations observed throughout the experimental period. Nevertheless, at the conclusion of the study, the dissolved oxygen stabilized, and the average values remained consistently above 4 mg/L.

The observed increase in electrical conductivity in the biofloc systems is probably due to the high concentration of decomposing organic matter generated by microbial flocs. Additionally, since water renewal only occurs through evaporation, the dissolved ions become more concentrated, resulting in increased conductivity and salinity. The *O. niloticus* species can endure salinity levels ranging from 0 to 18 g/L without any adverse effects on its survival, as reported by Schofield et al. (2011). The pH values did not show any significant differences between the treatments ($p > 0.05$), with a range from 7.1 to 7.9. This range falls within the ideal range for tilapia fingerlings (6.8 to 8), as stated by (Ahmad et al., 2017).

The variations in the concentration of total ammonia nitrogen (NAT) were observed in both the clear water and biofloc systems. The values ranged from 0.01 to 1.01 $mg \cdot L^{-1}$ in clear water and 0.01 to 1.06 $mg \cdot L^{-1}$ in biofloc. The low concentration of NAT in the biofloc system can be attributed to the addition of molasses, which promotes the growth of heterotrophic bacteria, keeping the ammonia concentration according to the acceptable range for Nile tilapia (Emerenciano et al., 2017; Pérez-Fuentes et al., 2016). Meanwhile, frequent water changes in the clear water system also contributed to the low NAT levels.

The concentration of nitrite was lower in the biofloc system, indicating the presence of nitrification. In contrast, the clear water system had a higher concentration of nitrite, likely due to a slower rate of nitrification (Widanarni et al., 2012). However, the observed concentration of nitrite was still acceptable, below 1 $mg \cdot L^{-1}$, as stated by Emerenciano et al. (2017). The clear water system showed no sedimentable solids, while the biofloc system had values ranging from 16 to 70 $mL \cdot L^{-1}$. According to Emerenciano et al. (2017), the recommended values are between 0.5 and 20 $mL \cdot L^{-1}$. In this study, the concentration of solids in the biofloc system was kept between 34.6 and 39.7 $mL \cdot L^{-1}$, which was higher than the recommended level.

Zootechnical performance

The use of hormones in biofloc and clear water systems did not affect final weight, weight gain, feed conversion, and specific growth rate ($p > 0.05$). These results align with those reported by Widanarni et al. (2012), who recorded a final weight for tilapia larvae ranging from 0.10 to 0.12 g, which is consistent with the findings of this study (ranging from 0.09 to 0.13 g). However, other studies have shown that biofloc can improve growth performance by serving as a natural and nutritious food source (Avnimelech, 2007), providing a good source of minerals, vitamins, and probiotics (Azim; Little, 2008).

Gonadal histology

Most of the fish in the BFT-H treatment exhibited undifferentiated characteristics, with few females and no males, while in the BFT-D treatment the fish remained undifferentiated throughout the study period (Figs. 1 and 2). According to Makino et al. (2009), histological examination of tilapia at 35 days showed immature gonads and a significant amount of connective and fibrous tissue. Carvalho and Foresti (1996) also conducted histological analyses of the gonads of tilapia that were fed less than a week after hatching and observed that fingerlings that did not receive the masculinizing hormone 17α-MT had fully differentiated gonads at 48 days for females and 68 days for males. The reference values provided by Costa e Silva et al. (2022) suggest that at 15 and 21 days after yolk absorption the histological analysis may not be an effective method for identifying male tilapia in BFT systems. However, at 28 days, the aceto-carmin squash method was successful in identifying males.

Based on this information, it is possible that in the current and in future studies the undifferentiated gonads observed may be due to the timing of the analysis or the method used. It may be necessary to use different methods, such as the aceto-carmin squash, or to wait until gonad differentiation has occurred in order to accurately identify the sex of the tilapia.

CONCLUSIONS

Tilapia post-larvae can be successfully reared in both biofloc and clear water systems without any negative impact on their zootechnical performance. The use of 17α-MT hormones for masculinization does not alter the physical and chemical water quality characteristics in either system. Therefore, such results showed that tilapia can be reared in alternative systems, which can increase the production of this species.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

The datasets generated during the current study are available from the corresponding author on reasonable request.

AUTHORS' CONTRIBUTION

Conceptualization: Silva UL, Gomes Júnior P, Falcon DR, Shinozaki-Mendes RA; **Supervision:** Silva UL, Gomes Júnior P, Falcon DR, Shinozaki-Mendes RA; **Investigation:** Gomes Júnior P, Falcon DR, Shinozaki-Mendes RA; **Validation:** Nascimento NF; **Data curation:** Nascimento NF; **Formal analysis:** Gomes Júnior P, Falcon DR, Shinozaki-Mendes RA; **Writing – original draft:** Silva UL, Nascimento NF; **Writing – review & editing:** Nascimento NF; **Final approval:** Silva UL.

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