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Comparison Between Environmentally Friendly Recirculation Systems with Biofilter, Green Water, Bioflocs, and Partial Water Exchange in the Cultivation of Nile Tilapia

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Abstract

With the intensification of aquaculture, sustainable farming systems are essential for the rational use of water. This study compared four rearing systems for juvenile *Oreochromis niloticus*: biofilter-based recirculation, green water, biofloc, and a partial water-exchange control, evaluating their effects on water quality and growth performance. Ninety-six juveniles (2.48 g) were stocked in sixteen 100-L aerated aquaria (four replicates per system) and reared for 60 days under controlled temperature. Water-quality variables and growth indices were analyzed using one-way ANOVA ($P<0.05$). The recirculation system provided significantly higher water hardness and calcium concentration than the other systems, as well as higher alkalinity than the control and green water systems, while maintaining nitrogenous compounds within safe limits. Fish reared in the recirculation and biofloc systems exhibited greater final weight, weight gain, specific growth rate, and an improved feed-conversion ratio compared to the control. The green-water system produced intermediate and non-significant responses. Survival, morphometric indices, salinity stress tolerance, and skin color parameters did not differ among treatments. Overall, the recirculation and biofloc systems improved the performance of juvenile tilapia under intensive rearing conditions, with the recirculation system providing better physicochemical water conditions and buffering capacity, making it the most robust and culture-efficient option..

Introduction

The continued growth of the human population, projected to reach approximately 10 billion people by 2050, demands a significant expansion of food production, with aquaculture playing a crucial role in this scenario (FAO, 2022). However, this growth cannot occur at the expense of environmental integrity. Conventional systems, although vital for supplying animal protein, are often associated with negative impacts, including water pollution and eutrophication (Ahmed & Turchini, 2021; Dong et al., 2022). Consequently, the development and adoption of innovative and sustainable strategies to mitigate these effects becomes imperative, ensuring ecosystem health, animal welfare, and social equity (FAO, 2022; Khanjani et al., 2023).

In this context, intensive technologies with greater resource use efficiency are gaining prominence. Recirculating Aquaculture Systems (RAS) are recognized

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for their high environmental control and water efficiency; however, energy consumption remains a concern (Ahmed & Turchini, 2021). Biofloc Technology (BFT) promotes nutrient recycling within the pond itself, converting nitrogen compounds into usable microbial biomass as supplemental feed, which can reduce feed conversion rates and production costs (Emerenciano et al., 2021; Khanjani & Sharifinia, 2020). Meanwhile, Green Water systems utilize microalgae communities and other microorganisms to improve water quality and modulate the presence of pathogenic bacteria (Ogello et al., 2023; Yang et al., 2020). Partial water exchange systems are also widely used, although they offer less control over environmental variables (Boyd & Tucker, 2012).

Promising technologies that employ traditional aquaculture principles with modern practices contribute to the sustainability of current aquaculture (Edwards, 2015). However, high stocking densities and large amounts of feed in intensive aquaculture pose risks and require regular monitoring of physicochemical parameters (Yusoff, 2024). It is necessary because poor water quality can stress fish, affecting their health, well-being, growth, reproduction, and even survival, compromising the success of cultivation and making production economically unviable (Zhang et al., 2025).

Despite these advances, a direct comparison between these systems remains lacking. When conducted with lower stocking densities, the adverse effect of high densities, which can mask the actual responses of the systems, is avoided (Li et al., 2021), leading to studies with reduced densities (e.g., 0.04 to 0.166 fish/L) (Munawarawanti et al., 2020; Matondang et al., 2022).

Given this gap, the present study aimed to compare the zootechnical performance and water quality of intensive systems considered more sustainable—recirculation with biofilter, bioflocs, and green water—with the partial water exchange system, evaluated simultaneously under controlled conditions.

Material and Methods

The experiment was conducted for 60 days, after approval by the ethics committee for the use of animals, CEUA-UFVJM, protocol 001/2021.

A total of 96 Nile tilapia juveniles, *Oreochromis niloticus*, weighing 2.48 g and with a total length of 50.2 mm, were randomly distributed in 16 aquariums with a volume of 100 L. Each aquarium received six animals at a density of 0.06 fish L^{-1}, with constant aeration (0.35 L air min^{-1}; dissolved oxygen of > 5.785 mg L^{-1}) and temperature controlled by thermostats (28.0±1°C) and a natural photoperiod in a recirculation system (water flow 1.1 L min^{-1}).

Fish were fed in three meals (8, 12 and 16 h) to apparent satiety (ad libitum), with commercial feed (2.5 mm) of composition: 360 g kg^{-1} crude protein (min.), 40 g kg^{-1} ether extract (min.), 55 g kg^{-1} crude fiber (max.), 120 g kg^{-1} mineral matter (max.), 20 g kg^{-1} calcium (min), and 120 g kg^{-1} moisture (max.). The offered and leftover feed were weighed to calculate the feed conversion rate (kg feed consumed kg. body weight $gain^{-1}$) and consumption at the end of the essay.

The animals were subjected to four different rearing systems: control, biofilter, green water, and bioflocs, with four replications each, in a completely randomized experimental design.

The control system was characterized exclusively by the recirculation of clean water, without the presence of any filter. In the recirculated water system (RAS), the water is recirculated through a biofilter composed of a substrate of limestone shells (1.3 cm in diameter), crushed stone (number 01), and gravel (2.1 cm in diameter) in a 1:1:1 ratio, contained in bags with 0.5 mm, mesh screens. The substrate made up 5% of the system volume. Before the start of the experiment, the biofilter was pre-matured for 30 days with commercial extruded feed (2–3 mm, 40% crude protein) administered daily. The amount of feed added was progressively increased from 5 to 15 g per cubic meter of water per day, divided into two daily doses, according to the methodology adapted from Sikora et al. (2020). The symbiotic DB Aqua (0.1 g m^{-3}) was added every 15 days, at the concentration suggested by the manufacturer. Aeration was maintained at a constant rate of 0.29 L of air min^{-1} to ensure the mixing of the water column and the suspension of particles. In the Green Water system, cell concentrations in water were maintained at around 92.5 x 104 mL^{-1}, with fortnightly checks, before cleaning the aquariums. When it was necessary to increase the concentration of cells, they were obtained from previously fertilized external tanks. Cell concentrations in the sample units were adjusted twice a week before cleaning the aquariums. The biofloc system was maintained at an approximate concentration of 500 mg L^{-1} of total suspended solids (Chutia et al., 2022; Serafini et al., 2025), measured in an Imhoff Cone. For maintenance, ammonia concentrations were measured twice a week and maintained at low levels by adding sugar with calculations following the methods described by Ebeling et al. (2006) and Avnimelech (1999). The biofloc was matured for 90 days using sugar-based fertilization and commercial feed (C: N 20:1) (Aboseif, et al., 2022), from which the inoculum added to the sample units was obtained.

Every seven days, the aquariums were cleaned by siphoning, renewing 10% of the water in the system. Weekly, before cleaning (8 h) pH and TDS (g L^{-1}) (Instrutherm mod. pH-1500), the conductivity (mS cm^{-1}) (model CD 850 Instrutherm), and the turbidity (NTU) (Tecnal TB 1000 P), were measured. The temperature (°C) and dissolved oxygen concentration (mg L^{-1}) (8 and 15 h) were measured using an oximeter (YSI Ecosense DO200A).

The alkalinity (mg L^{-1}), non-ionized ammonia (mg L^{-1}), nitrite (mg L^{-1}), nitrate (mg L^{-1}), hardness (mg L^{-1}), phosphate (mg L^{-1}), and calcium (mg L^{-1}) were monitored according to APHA (2016).

Samples were collected every fifteen days throughout the experiment to determine the approximate composition of the bioflocs when the protein, lipid, ash, and moisture content were analyzed (AOAC, 2006). Protein was estimated from the nitrogen concentration obtained by the Kjeldahl method (nitrogen x 6.25). Lipid content was determined by Soxhlet extraction with the solvent ether (boiling point, 40–60 ◦C). The moisture percentage was determined by drying the samples at 105°C until constant weight, and the ash percentage was obtained from the samples in a crucible and subjected to a muffle furnace at 600°C for three hours.

At 60 days, the weight of the fish (g) was measured with an analytical balance with a precision of 0.01 g, and the total length, standard length (cm), and body height (cm) were measured with a digital caliper (Starrett) with a precision of 0.02 mm. The fish were counted and the survival rate (%) obtained. From these records,

Weight gain (g) = final average weight—initial average weight

The biomass (g) = sum of the weights of the individuals in the aquarium,

Biomass gain (g) = final biomass—initial biomass

The Fulton condition factor (K), = 100 × (total length weight^{-3})

Specific growth rate (SGR) (%)= 100 × ((Ln final weight – Ln initial weight) (time between weightings)$^{-1}$)

The ratios (%) of height with total length and standard length were also calculated.

After the final biometry (24 h), two fish from each experimental unit were subjected to acute salinity stress (40 g L^{-1} of NaCl), and the duration of tipping in the water column was evaluated.

At the end of the experiment, eight juveniles of each treatment were randomly sampled, and the color in the base of the pectoral fin on the left side was measured using a Minolta CR400 colorimeter.

The skin colors were quantified according to the CIE L*, a*, and b* (coordinates Huntellab CIELab), where L* represents the luminosity or incidence of light (black= 0, white= 100); a* measures the red-green dimension with positive values for redness and negative for green; and b* represents the yellow-blue dimension with positive values for yellow and negative for blue. The chromaticity values of a* and b* were transformed into specific chromatic attributes for calculating the color saturation, called chroma (C^*_{ab}). The equation used for this transformation was: $C^*_{ab}=(a^{*2}+b^{*2})^{0.5}$. The Hab, the amplitude angle expressed in degrees, was also calculated (Hab = arctan (b*/a*)).

To determine the bromatological composition of the bioflocs, samples were collected every 20 days, dried in a forced-air oven at 55°C until a constant weight was achieved, and subsequently ground. Analyses were performed in triplicate according to the official AOAC (2006) methods. Crude protein content was determined by the Kjeldahl method (AOAC 984.13), using a factor of 6.25 to convert total nitrogen to crude protein. Ether extract (total lipids) was quantified by continuous extraction in a Soxhlet apparatus with petroleum ether (AOAC 920.39). Crude fiber was determined by the Weende method (AOAC 962.09), and ash content by incineration in a muffle furnace at 550°C for 5 hours (AOAC 942.05). All results are expressed as a percentage of dry matter (% DM).

All the data were subjected to normality and homoscedasticity tests to evaluate the effects of the systems. The data for each parameter were grouped and subjected to one-way ANOVA ($P<0.05$), and the means were compared using the Tukey test. Statistical analysis was performed using the SAS program (SAS Institute, Inc., Cary, NC, USA).

Results

Water quality parameters are shown in Table 1. pH, alkalinity, hardness, calcium, and conductivity differed among systems. The control had a slightly higher pH (6.12±0.345) than the other treatments, although they were remarkably close. Alkalinity was higher in the biofilter (16.21 mg L^{-1}) and biofloc (9.54 mg L^{-1}) systems than in the control (9.06 mg L^{-1}) and green water (8.94 mg L^{-1}) systems. The biofilter system also had the highest level of hardness (159.37 mg L^{-1}) and calcium (111.55 mg L^{-1}), and the biofloc system had the highest conductivity (0.501mS cm^{-1}). The values of ammonia, nitrite, nitrate, phosphate, temperature, dissolved oxygen, turbidity, and total suspended solids did not differ**.** The water in all systems became turbid during the period.

The values of final weight (39.35 g; 41.22 g), weight gain (35.41 g; 36.97 g), and specific growth rate (4.37%; 4.46%) of the animals subjected to the biofilter and bioflocs, respectively, were higher than those observed in the animals subjected to the control system (Table 2). The values of these same parameters of the fish subjected to the green water system were intermediate, not differing from the control, the biofilter, or the bioflocs. Among the parameters measured, including survival rate, feed consumption, biomass, biomass gain, final total length, final standard length, final height, height-to-total length ratio, height-to-standard length ratio, Fulton condition factor, and tumbling time, there were no significant differences.

The apparent feed conversion of the control system was higher than that of the other systems. The parameters, survival, consumption, biomass, biomass gain, total length, standard length, height, height/total length and height/standard length, Fulton condition factor, and tumbling time did not differ. Juveniles in clearer waters, biofilter and control, showed slightly more agitated behavior.

The fish's skin coloration showed differences between the treatments detected by the coordinates b* and C (Table 3).

The bromatological composition of bioflocs used in the cultivation of Nile tilapia is shown in Table 4.

Discussion

Water Parameters

The physicochemical parameters of water are a limiting factor in preserving the health of aquaculture species (Boyd & Tucker, 2012). In this experiment, water parameters remained within the comfort range of the *O. niloticus* in a static system with partial renovation water (Elnady et al., 2015), recirculating (Gullian-Klanian & Arámburu-Adame, 2013; Fleckenstein et al., 2018; Lailiyah et al., 2023), green water (Al-Hafedh & Alam, 2005; Alam & Al-Hafedh, 2006; Suárez-Puerto, 2021) and biofloc (Martins et al., 2017; Fleckenstein et al., 2018; Suárez-Puerto, 2021; Zablon et al., 2022) tanks, presenting good conditions for the development of tilapia juveniles in the tested treatments. However, there were differences in water quality sufficient to promote differences in the development of fish.

Temperature and oxygen, parameters of outstanding importance in Nile tilapia physiology (Abd El-Hack et al., 2022), were within the ideal range and were similar for all cultivation systems, providing good conditions and showing these parameters were not decisive in the differences found.

Ammonia and nitrite, which are considered toxic, also were within a comfort range for the species and were similar to each other, as were phosphate and nitrate, which are low in toxicity but are indicators of eutrophication of the environment. Therefore, these parameters added to temperature and oxygen, indicating good cultivation conditions.

Table 1. Mean values (±standard deviation) of water quality parameters of Nile tilapia (*Oreochromis niloticus*) culture to different rearing environments for 60 days

Parameters	Control	Biofilter	Green Water	Bioflocs
pH	6.12±0.35^{a}	5.90±0.47^{b}	6.06±0.26^{ab}	5.96±0.24^{ab}
Alkalinity (mg L^{-1})	9.06±2.95^{b}	16.21±5.87^{a}	8.942±2.19^{b}	9.536±2.54^{a}
Calcium (mg L^{-1})	22.40±12.72^{b}	111.55±51.06^{a}	32.86±19.75^{b}	51.72±29.13^{b}
Hardness (mg L^{-1})	75.71±21.96^{b}	159.37±62.69^{a}	79.18±29.20^{b}	110.48±37.20^{b}
Ammonia (mg L^{-1})	0.06±0.02^{a}	0.05±0.01^{a}	0.06±0.02^{a}	0.07±0.05^{a}
Nitrite (mg L^{-1})	0.15±0.03^{a}	0.03±0.01^{a}	0.09±0.26^{a}	0.11±0.09^{a}
Nitrate (mg L^{-1})	49.75±24.01^{a}	36.33±16.60^{a}	45.35±17.38^{a}	48.74±22.55^{a}
Phosphate (mg L^{-1})	1.221.32^{a}	0.85±0.78^{a}	1.36±0.69^{a}	2.81±2.05^{a}
Conductivity (mS cm^{-1})	0.10±0.07^{b}	0.22±0.10^{ab}	0.31±0.10^{ab}	0.51±0.19^{a}
Turbidity (NTU)	158.48±37.78^{a}	126.04±58.66^{a}	184.56±71.20^{a}	210.18±48.13^{a}
TDS (g L^{-1})	0.07±0.05^{a}	0.13±0.07^{a}	0.19±0.05^{a}	0.29±0.11^{a}
Temperature (ºC) (AM)	23.58± 1.29^{a}	23.70±1.00^{a}	23.60±1.01^{a}	24.01± 0.92^{a}
Temperature (ºC) (PM)	27.36±1.23^{a}	26.71±1.13^{a}	27.54±1.20^{a}	27.61±1.30^{a}
DO (mg L^{-1}) (AM)	6.86±0.71^{a}	7.25±0.80^{a}	7.05±0.66^{a}	6.76±0.72^{a}
DO (mg L^{-1}) (PM)	5.82±1.08^{a}	5.98±1.11^{a}	5.78±1.04^{a}	5.80±1.05^{a}

Means followed by different letters on the same line differ from each other, by Tukey's test, at 0.05 probability. Legend: pH= Hydrogen point; TDS=Total dissolved solids; DO=Dissolved oxygen.

Table 2. Mean values (±standard deviation) of performance of Nile tilapia (*Oreochromis niloticus*) cultured to different rearing environments for 60 days

Parameters	Control	Biofilter	Green Water	Bioflocs
Survival (%)	91.7±9.6^{a}	95.8±8.3^{a}	100.0±0.0^{a}	100.0±0.0^{a}
Weight (g)	26.60±14.77^{b}	39.35±14.81^{a}	30.66±10.89^{ab}	41.22±14.14^{a}
Weight gain (g)	23.25±14.98^{b}	35.41±16.07^{a}	28.18±10.89^{ab}	36.97±15.66^{a}
Consumption (g)	276.7±70.7^{a}	272.2±92.5^{a}	242.1±84.4^{a}	273.6±143.4^{a}
Feed conversion rate	2.0±0.3^{a}	1.3±0.1^{b}	1.3±0.3^{b}	1.2±0.3^{b}
Biomass (g)	141.5±52.9^{a}	202.9±61.2^{a}	184.0±43.5^{a}	224.6±62.3^{a}
Biomass gain (g)	115.6±57.9^{a}	164.0±56.5^{a}	153.3±45.8^{a}	183.9±67.3^{a}
Total length (mm)	11.07±1.90^{a}	12.27±1.70^{a}	11.56±1.58^{a}	12.42±1.52^{a}
Standard length (mm)	9.02±1.60^{a}	10.04±1.25^{a}	9.32±1.10^{a}	10.31±1.37^{a}
Height (mm)	2.75±0.60^{a}	3.05±0.49^{a}	2.94±0.49^{a}	3.28±0.45^{a}
Height/Total length (%)	25.1±3.1^{a}	25.1±2.4^{a}	25.4±2.3^{a}	26.3±2.1^{a}
Height/Standard length (%)	30.9±3.4^{a}	30.6±2.7^{a}	32.0±4.3^{a}	31.6±2.5^{a}
SGR (%- day1)	3.61±1.03^{b}	4.37±0.87^{a}	4.09±0.61^{ab}	4.46±0.81^{a}
Fulton (K)	3.27±0.43^{a}	4.18±3.29^{a}	3.86±1.66^{a}	3.66±0.46^{a}
Tipping time (min.)	135.8±87.1^{a}	116.4±51.3^{a}	96.8±42.1^{a}	101.3±55.7^{a}

Means followed by different letters on the same line differ from each other, by Tukey's test, at 0.05 probability. Legend: SGR= specific growth rate.

The highest total ammonia concentration (0.07 mg L^{-1}) was lower than those observed in Nile tilapia culture in control (0.64 to 1.8 mg L^{-1}) (Mirzakhani et al., 2019; Haraz et al., 2023), bioflocs (0.32 to 6.4 mg L^{-1}) (Mirzakhani et al., 2019; Haraz et al., 2023), and recirculation system (0.010 to 0.107 mg L^{-1}) (Wambua et al., 2021), when the concentrations were considered adequate for the species.

Ammonia and nitrite are toxic under different environmental conditions and can severely affect fish physiology, leading to significant production losses. Therefore, reducing their concentrations in culture is necessary, either by oxidation, leading to nitrate, a less toxic form, or by removing these compounds (Robles-Porchas et al., 2020; Sarosh et al., 2024). Nile tilapia should be maintained below 0.1 mg l^{-1} un-ionized ammonia (El-Shafai et al., 2004) or 2 mg l^{-1} total ammonia to avoid tissue damage to the gills, liver and kidney, which may be preludes to disease susceptibility (Benli et al., 2008). For Nile tilapia *O. niloticus*, the median lethal concentration (LC_{50}) was 1.46 mg L^{-1} of non-ionized ammonia at 24 and 48 h of exposure and 0.98 mg L^{-1} at 96 h (Evans et al., 2006).

The highest nitrite concentration among the environments was 0.15 mg L^{-1}, below the maximum nitrite value considered adequate to Nile tilapia reared in a recirculating system with biofilter (0.59 a 0.72 mg L^{-1}) (Ridha & Cruz, 2001), control (0.43 mg L^{-1}) and bioflocs (0.14 to 0.24 mg L^{-1}) (Putra et al., 2020). However, smaller tilapia (4.4 g) are more tolerant to nitrite than larger ones (90.7 g), and the addition of chloride (calcium chloride or sodium chloride) increases the fish's tolerance to nitrite (Atwood et al., 2001). In day by day, the nitrite problems can be significantly reduced by avoiding overcrowding and overfeeding, performing regular water changes, maintaining aeration through the biological filter, by microalgae, through bioflocs, electrochemical oxidation, and adding salt chloride (Ciji & Akhtar, 2019).

As for nitrates, concentrations below 500 mg L^{-1} are recommended in the culture of juvenile tilapia to ensure optimal health and growth conditions. Values much higher than those observed (49,75±24,01 mg L^{-1}) in this experiment (Monsees et al., 2017).

Phosphate levels (0.85 to 2.81 mg L^{-1}) in this experiment were higher than those typically found in Nile tilapia culture. Concentrations of 0.16±0.05 mg L^{-1} of total phosphorus and 0.09±0.03 mg L^{-1} of orthophosphate are found in concrete tanks of Nile tilapia farmers (Moustafa et al., 2020), and about 0.35 to 0.60 mg L^{-1} of orthophosphate, in bioflocs systems (Aboseif et al., 2022). On the other hand, these values were lower than those observed in tilapia cultivation in an aquaponics system, where both total phosphorus (TP) and phosphate PO4-3 increased continuously due to the addition of feed (27.9 to 68.7 mg L^{-1} of total phosphorus and 20.4 mg to 61.4 L^{-1} of phosphate, in the second and fourth weeks, respectively, when tilapia growth was observed (Liang & Chien, 2013). The fact that Nile tilapia can adapt and live even in environments with high phosphate levels, such as a hypereutrophic urban lake, a eutrophic rural reservoir, and a hypereutrophic aquaculture property (Shimada Borges et al., 2013). Therefore, the results indicate that the oxygen level, food management, and periodic cleaning were adequate to maintain good water conditions.

Biological Filter

The water from the biofilter system had higher hardness and calcium concentration than the control, green water, and biofloc systems, and alkalinity was similar to that of the biofloc system and higher than that of the control and green water systems.

Table 3. Mean values (±standard deviation) of skin color parameters of Nile tilapia juveniles submitted to different culture systems for 60 days

Parameters	Control	Biofilter	Green water	Bioflocs
a* (chromaticity)	29.0±13.4^{a}	34.8±0.9^{a}	33.3±1.0^{a}	30.7±1.5^{a}
b* (chromaticity)	15.2±8.0^{ab}	15.9±2.0^{a}	10.1±3.5^{ab}	9.5±1.1^{b}
L (luminosity)	14.4±6.7^{a}	13.0±0.9^{a}	11.6±0.9^{a}	10.1±0.7^{a}
C (chroma)	35.8±2.2^{b}	38.3±1.6^{a}	34.9±1.6^{b}	32.1±1.5^{c}
Hab (tone)	29.7±27.0^{a}	24.5±2.3^{a}	16.7±5.5^{a}	17.2±1.8^{a}

Means followed by different letters on the same line differ from each other, by Tukey's test, at 0.05 probability. Legend: a* intensity of red (+) and green (-); and b* intensity of yellow (+) and blue (-); L* measures the luminosity and varies from 100 (one hundred) for perfectly white surfaces to 0 (zero) for black; C represents the saturation, and h is the hue angle.

Table 4. Average values (±standard deviation) of the bromatological composition of bioflocs over 60 days in the maintenance of juvenile Nile tilapia

Average	Composition (%)	CV
Crude Protein	31.3±3.5	11.146
Ether extract	5.3±0.3	5.873
Crude Fiber	43.7±3.23	7.401
Ash	19.3±0.9	4.739

CV-Coefficient of variation.

The fish from the recirculation system presented weight, weight gain, and SGR as equal to those observed in the bioflocs system and higher than those obtained in the control and green water systems. They also had a lower feed conversion than that observed in the control system, showing greater efficiency.

The biofilter recirculation system is a widely used sustainable system (Wicaksono et al., 2024), which can have different compositions (Hassan et al., 2022). It is known to maintain good water quality and increase productivity, as observed for Nile tilapia (Wicaksono et al., 2024), carp *Cyprinus carpio* (Hassan et al., 2022), rainbow trout *Oncorhynchus mykiss* (Nędzarek et al., 2022), *Rhamdia quelen* (de Souza et al., 2024), among others.

One of the reasons for the good results of this system is the nitrification process in the biofilter (Wicaksono et al., 2024; de Souza et al., 2024) and the practice of alkalization (de Souza et al., 2024), as in this experiment. Alkalinity indicates the water's ability to buffer variations in water quality, stabilize the environment, avoid stress for fish, and provide greater growth (Boyd & Tucker, 2012). For alkalization, with improved water quality and increased production, different alkalizing agents have been used, where calcium carbonate provided good water stability over time, good nitrification efficiency, and, due to calcium, increased hardness (de Souza et al., 2024), which has a protective effect on fish (Copatti et al., 2019). However, there is a limit to the concentration of calcium carbonate, which can hurt tilapia growth (Martins et al., 2017), even when this carbonate comes from pieces of shells. When the proportion of shells/gravel was 3:7, there were better nitrification rates in the cultivation of Nile tilapia than in higher concentrations of shells (Duarte et al., 2023).

Beyond the benefits mentioned, the superior results of the biofilter system can be further understood through a synergy of stabilizing mechanisms initiated by the calcium carbonate substrate. This component acted as a double source of benefits: it supplied Ca^{2+} ions to the water and served as an alkaline reserve. Consequently, the system not only maintained a higher alkalinity (buffering capacity), ensuring pH stability, but also raised the concentration of dissolved calcium to adequate levels. This combination is physiologically crucial. While calcium from the feed meets structural demands, calcium from the water is fundamental for efficient osmoregulation (Paterson, 1978; Flik and Verbost, 1995). As demonstrated by Copatti et al. (2019), Ca^{2+} in the water reduces the permeability of the gill epithelium, decreasing the passive loss of ions and, therefore, the energy cost of the Na^+/K^+-ATPase pump. In this way, the biofilter simultaneously mitigated acid stress (via buffering) and osmotic stress (via calcium supply), releasing a significant portion of metabolizable energy for somatic growth and resulting in the best zootechnical performance observed.

Therefore, the good results obtained in this experiment's recirculation system with biofilter can be explained by using broken shells composed of calcium carbonate, which made up the substrate, in adjusted quantities.

Bioflocs System

Similarly observed in the biofilter system, weight, weight gain, and SGR were higher in tilapia juveniles with bioflocs compared to the control and green water systems. Bioflocs improve the quality of culture water and reduce the occurrence of pests and diseases (Li et al., 2025). However, the greater weight gain in the biofloc system has been attributed to the satisfactory nutritional status of the fish (Azim & Little, 2008; Mirzakhani et al., 2019; Khanjani & Sharifinia, 2020), since this system provides food for tilapia easily and continuously (Ekasari et al., 2015), providing additional proteins that contribute to increased growth (Suárez-Puerto et al., 2021), as well as a wide variety of nutrients, such as lipids, ash and micronutrients (fatty acids, amino acids, minerals) (Khanjani et al., 2023).

Bioflocs are also considered probiotic (Khanjani et al., 2023), resulting in improvements in intestinal morphology and immune responses (Mirzakhani et al., 2019). The bioactive compounds in bioflocs possess antibacterial/antioxidant properties (Li et al., 2025), which minimize bacterial infections, increase resistance to saline stress, and lead to higher survival rates (Ekasari et al., 2015), thereby improving welfare and production (Azim & Little, 2008).

The SGR values of tilapia in the biofloc system were higher than in the control, as observed for juvenile tilapia (Mirzakhani et al., 2019; Eid et al., 2021; Zablon et al., 2022). The increase in SGR is observed even when the biofloc reuse rate increases (Figueroa-Espinoza et al., 2022). However, sometimes differences may not occur, as observed for tilapia larvae (Ekasari et al., 2015). The feed conversion rate was also optimized and was lower than in the control system. The lower feed conversion rate of tilapia was attributed to the bioflocs as food, which reduces the need for feed (Azim & Little, 2008; Mirzakhani et al., 2019; Zablon et al., 2022).

Thus, bioflocs improved feed conversion and growth not only through supplemental nutrition but also by optimizing the use of the main feed, reducing latent immunological costs, and improving water quality.

Bromatological Composition of Bioflocs

Bioflocs are usually considered to be of good nutritional quality due to their high protein content, in addition to lipids, vitamins, and micronutrients (Khanjani et al., 2023), which increases enzymatic activity, immune response, and tilapia yield (Long et al., 2015).

In this study, the crude protein level average (31.3±3.5%) of the bioflocs was adequate for tilapia.

These values were within the range observed by Khanjani and Sharifinia (2024) (28.7%) and Lima et al. (2021) (31.31 to 32.33%), below that observed by Long et al. (2015) (41.13%), Azim and Little (2008) (37.93 to 38.41%) and Hisano et al. (2019) (38.8 to 39.7%), but above that observed by Martins et al. (2017) (13.20 to 17.85%), da Silva et al. (2019) (23.45 to 24.69%), Mabroke et al. (2021) (19.6 to 23.1%) and Durigon et al. (2020) (17.39%), cultures where tilapia were fed with feed and showed satisfactory growth.

The level of lipids (5.3±0.3 %) found in bioflocs of this experiment presented above those mentioned by Mabroke et al. (2021) (1.28 to 1.85%), Martins et al. (2017) (2.11 to 2.65%), da Silva et al. (2019) (1.02 to 3.5%), Durigon et al. (2020) (1.22%), Long et al. (2015) (1.3%), Azim and Little (2008) (3.16 to 3.23%), Lima et al. (2021), (2,02 to 2.11%), and Khanjani and Sharifinia (2024) (2.4%).

The biofloc ash content (19.3±0.9%) in this experiment was higher to that observed by Lima et al. (2021) (10.33 to 10.78%), but fewer than that observed by Martins et al. (2017) (35.02 to 44.03%), da Silva et al. (2019) (37.55 to 39.56%), Mabroke et al. (2021) (43.66 to 47.99%), and Khanjani and Sharifinia (2024) (32.8%). The lipids value and ash were found in cultures where tilapia were fed with feed and showed satisfactory growth.

The biochemical composition of bioflocs and their nutritional value vary according to environmental conditions and the stage of development, presenting quite diverse compositions and relationships (Khanjani et al., 2023). Despite presenting variations in their composition, when used as food, bioflocs usually provide an improvement in feed conversion or an increase in tilapia yield, in addition to affecting the composition of juvenile Nile tilapia (Lima et al., 2018). Finally, the composition of the bioflocs was within that found by several authors, and the juveniles showed good growth, indicating that the bioflocs were suitable for feeding juvenile Nile tilapia.

Green Water

The green water system showed a better feed conversion rate than the control, and the parameters, weight gain, and SGR were intermediate between the control and the biofloc and biofilter recirculation systems.

The green water system is created by intensifying the cultivation of local or introduced phytoplankton, which, despite the name, can have different colors. Microalgae are intended to improve water quality by removing nitrogenous substances, increasing oxygenation, dispersing and attenuating light, and inhibiting bacterial activities, in addition to stimulating digestion and improving direct and indirect nutrition of larvae, increasing survival, growth, and decreasing stress levels (Palmer et al., 2007). *Chlorella vulgaris* and *Scenedesmus obliquus* improved water quality, immune response, development, and body composition compared to controls of juvenile Nile tilapia (Jung et al., 2017). However, the effects depend on factors such as the feeding habits of the fish throughout their growth and the management of the producers, processes that control the dynamics of the algae population, and the efficiency of the food chains (Neori, 2011), which explains the partial improvement observed in this experiment, in comparison the control. It is also worth noting that although the green water system is predominantly composed of microalgae, it is also composed of bacteria, protozoa, and zooplankton (Neori, 2011), which makes it very close to the biofloc system and is currently sometimes called the autotrophic biofloc technology system (Jung et al., 2017). Comparing the systems, green water (autotrophic) and biofloc (heterotrophic) positively affect the growth performance of Nile tilapia, in addition to presenting excellent water purification capacities compared to the control group. However, better growth and wastewater treatment capacity results were obtained in the biofloc system (heterotrophic system) than in the green water system (autotrophic) (Kim et al., 2020). This intermediate growth can be attributed to the fact that fish have difficulty digesting microalgae, as these contain a variety of unfavorable components, such as cell wall fibers and antinutritional factors, which pose a challenge to fish digestion (Lu et al., 2023), thereby justifying the intermediate results.

Skin Color

The chromatic parameters, chroma, which indicates the intensity of the color, and chromaticity, related to the saturation of the colors, presented higher values in the fish raised in the system with a biofilter, where the water was clearer, compared to the fish raised in the biofloc system, characterized by more turbid water. In the control and green-water systems, the values of these parameters were intermediate. This variation in the results reflected the trend observed in the water turbidity between the systems, although such a difference was not statistically significant.

Differences in skin coloration and behavior of Nile tilapia have long been observed in response to variations in environmental color and turbidity (Luchiari et al., 2007; Opiyo et al., 2014; Rebouças et al., 2014; Ariyomo et al., 2024). Juvenile Nile tilapia (*O. niloticus*) raised in tanks with clear bottoms presented lighter coloration and more aggressive behavior (Opiyo et al., 2014), behavior similar to that observed in fish raised in clearer waters in the biofilter system of this experiment. Furthermore, juveniles Nile tilapia demonstrates behavioral hierarchies when competing for shelter, with a preference for yellow structures over other colors, such as blue, green, white, and red. This preference can be attributed to the similarity of yellow with the characteristics of the natural environment of these fish (Luchiari et al., 2007).

Fish farming in the biofloc system has several effects on physical and biological parameters. In the case of goldfish (*Carassius auratus*), the biofloc system, compared to the clear water system, presents greater water turbidity, which resulted in the intensification of skin pigmentation without, however, affecting the development of the animals (da Cunha et al., 2020). On the other hand, the increase in turbidity caused by the presence of clay is associated with a reduction in the development and survival rate of red tilapia (*Oreochromis* sp.) (Ardjosoediro & Ramnarine, 2002).

At high concentrations of bioflocs, turbidity reduces visibility and negatively impacts feed intake, which can compromise tilapia growth. However, by understanding the composition and interactions in bioflocs, it is possible to adjust the system to mitigate these effects and achieve satisfactory results since bioflocs also serve as a food source for tilapia (Azim and Little, 2008).

Therefore, the diversity of color and turbidity of the environment can influence both the coloration and behavior of fish, which exhibit phenotypic plasticity.

Finally, it is concluded that the variation in skin color observed in juvenile tilapia is associated with the turbidity of the water in each system, which was adequate for the species.

Stress Test

The tumble times of fish in the four treatments were statistically equal. They may be related to the good farming conditions provided by the systems, as well as to the tolerance capacity of Nile tilapia to variations in the salinity of the breeding water (Souza et al., 2019; Fuadi et al., 2021; Mohamed et al., 2021; Seale et al., 2024).

Environmental salinity influences the life history of fish, affecting their distribution and diversity. Changes in salinity affect physiological processes, including metabolism, nutrition, reproduction, and growth, and fish can be conditioned by environmental parameters (Seale et al., 2024). Although Nile tilapia is tolerant, several factors interfere with a greater or lesser capacity for tolerance to salinity, including hereditary genetic factors (Yamaguchi et al., 2018), improvement, and genetic selection (El-Leithy et al., 2019; Yue et al., 2024), the interaction between water quality parameters (Xing et al., 2022), the use of immunostimulant feed additive (El-Leithy et al., 2019), in addition to the development stage.

Salinity tolerance is a characteristic of tilapias where other species or hybrids, in addition to Nile tilapia, may present greater capacity (Seale et al., 2024). Is the case of *Oreochromis mossambicus*, *Oreochromis aureus*, *Oreochromis spilurus*, *Oreochromis urolepis hornorum*, *Sarotherodon galilaeus*, and *Coptodon zillii* in addition to hybrids derived from salinity-tolerant tilapia species, which have been used in aquaculture production in brackish water and seawater (Yue et al., 2024).

Nile tilapia *O. niloticus* (1.62 g) can be raised in a recirculation system for 45 days at salinities up to 7‰ without compromising growth despite presenting gill infection (De Azevedo et al., 2015). Nile tilapia (93.8 to 280 g) grown in bioflocs for 90 days at a salinity of 16‰ showed good performance and high survival rates; however, stress was observed, indicated by increased glucose levels and changes in body integrity (Souza et al., 2019). At higher salinity concentrations, 30‰, Nile tilapia (11 to 13.5 cm, approximately 40 g) began to exhibit erratic swimming and slow opercular beating, indicating severe stress. In the salinity to 40‰, tilapia can survive up to 1 hour (Fuadi et al., 2021). Studies with Nile tilapia (10.2 to 12 cm and weight of 25.5 to 26.15 g) have shown that mortality increases with salinity (mortalities of 3.7, 7.8, 19.5, 25, 30.7, 39.8, 49.6, 55.3, 60.5, 65.9 and 77.3%, for salinities of 6, 12, 16, 18, 20, 22, 24, 26, 28, 30, 32 and 34‰ respectively) (El-Leithy et al., 2019). Therefore, although aquatic organisms have self-regulation mechanisms to mitigate impacts caused by increased salinity, high concentrations can disrupt energy metabolism, induce inflammation, and negatively affect normal growth and development (Li et al., 2024).

The similarity of tumbling times can be explained by the fact that the tilapia juveniles in this experiment were obtained from the same batch and subjected to good cultivation conditions before being subjected to the salinity stress test.

Conclusion

In summary, the biofilter system showed the best productive performance and the greatest stability in water quality, indicating a higher potential to operate at high stocking densities or with longer intervals between maintenance events. The biofloc system achieved zootechnical performance similar to that of the biofilter, although with less favorable water quality parameters. The green-water system, in turn, showed intermediate results between the partial water-renewal system and the more intensive systems (biofloc and recirculation). Despite these differences, all systems proved to be viable for the production of Nile tilapia juveniles, allowing producers to choose the technology that best fits their objectives and management conditions.

Ethical Statement

Approved by the ethics committee for the use of animals, CEUA-UFVJM, protocol 001/2021.

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Author Contribution

Adriana de Fátima Rocha and Marcelo Mattos Pedreira conceived the study, conducted, wrote the original version of the manuscript, and edited the article. Talita Andrade Ferreira and Cleube Andrade Boari assisted in the laboratory experiment, helped with the methodology and writing.

Conflict of Interest

The authors declare no potential conflict of interest.

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